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Current and Proposed Regulations

Chairman: ALBERT MINKS

INSECT BEHAVIOR-MODIFYING CHEMICALS: THE STATUS OF CURRENT AND PROPOSED REGULATION IN THE UNITED STATES

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ABSTRACT

EPA endeavors to promote microbial and biochemical pesticides as effective, environmentally harmonious alternatives to certain conventional chemicals. EPA has illustrated its commitment to simplifying the registration process of biological agents by establishing a reduced set of data requirements, by expediting scientific reviews of these products and lessening processing times. EPA is studying internal processes and the policies applied to microbial and biochemical pesticide registration applications. From a regulatory perspective, the registration process has been simplified. Still EPA seeks to find additional ways to streamline these registrations. Interested parties must recognize that the Agency's most significant constraint is the need to base all regulatory decisions upon sound scientific evidence. Analysis of individual classes of semiochemicals may allow the Agency to further adjust data requirements for specific classes of biochemicals. EPA has performed the first of such analyses, which is currently under review. The Agency extends a challenge to the scientific and academic communities as well as the regulated industry to share resources, expertise and ingenuity to identify and scientifically justify alternatives to the current registration process.

In the United States (U.S.), as in other countries, there is now a public focus on food safety and the environment. The use of pesticides is no longer viewed as a strictly beneficent activity. Pesticides are scrutinized in relation to their effects on nature as well as humans, both as applicators and as consumers of the food products treated. The U.S. Environmental Protection Agency (EPA) serves as the American public's quality control agent for pesticide use. Our public relies on the EPA to evenly and objectively review scientific data, and to assess both the benefits and the risks associated with product use before granting a registration. The EPA develops and maintains the confidence of the public, researchers and registrants by subjecting all candidates for registration to consistently rigorous submission requirements and review processes. Public confidence in the regulatory process is critical to the successful marketing and use of pesticide products, both

biological pesticides as well as conventional chemical pesticides. These actions are conducted under the authorization of the Federal Insecticide, Fungicide and Rodenticide Act (FIFRA).

Over the last year, EPA has developed an Agency strategy for accomplishing its human health and environmental protection responsibilities. The purpose of the strategy is to articulate clear, broad goals which we can use to evaluate the significance of our current activities, to identify important new efforts we should be pursuing and overall to realign Agency programs to achieve our strategic goals. The universe of possible environmental protection activities which EPA might undertake is far greater than resources available to accomplish them. The American public, with all its many constituencies and interest groups, makes enormous and varied demands on us daily. Our own staff is also very capable of finding new tasks to undertake. Though we are a "can do" sort of place, we have found there are significant limits to our abilities to meet these external and internal demands. The Agency strategy is an attempt on our part to prioritize these demands and to keep us focussed on those activities which will accomplish the most in terms of human health and environmental protection.

In the case of pesticides, one of our broad goals is to use our regulatory process to foster the development, registration, marketing and use of safer pesticides. Achieving this goal is going to be difficult--just effecting the necessary cultural shift within the Agency is a major challenge. We are trying to change from an organization which rather neutrally evaluated pesticides and refrained from taking an advocacy position to an organization that actually seeks ways to support safer products and to influence both pesticide producers and users to do likewise.

Nevertheless, these internal changes at EPA are taking place. And as they do, they are creating new opportunities to make improvements in EPA's regulatory program for biological pesticides. What I'd like to do this morning is to describe briefly EPA's regulatory authority in this area, our early efforts to promote registration of biologicals, our current requirements and then to conclude with opportunities we see for change.

REGULATORY AUTHORITY

In the U.S., the Environmental Protection Agency's Office of Pesticide Programs (OPP) is charged with implementing FIFRA. That Act requires that before any person in any state or foreign country can sell or distribute any pesticide in the U.S., they must obtain a registration (or license) from the EPA. For the purposes of FIFRA, a pesticide is defined as any substance or mixture of substances intended for preventing, destroying, repelling or mitigating any pest, or intended for use as a plant regulator, defoliant or desiccant. Because the

key to a compound's regulatory status is the intent of its use, as a rule biochemical pesticides, including insect pheromones, fall under the jurisdiction of FIFRA. For example, a pheromone used to attract insects for the purpose of mitigating a pest population is a pesticide.

On the other hand, the EPA recognizes that certain uses of semiochemicals are not pesticidal and so are not subject to regulation by FIFRA. Among those are semiochemicals which are utilized to attract an insect species solely for survey or detection purposes. EPA also has authority to grant exemptions from FIFRA requirements if regulation is not needed to ensure that a pesticide poses no unreasonable adverse effects. In practice, EPA has exempted those pheromone products which are labeled exclusively for use in traps in which they are the sole active ingredient, and where their use will not significantly increase the concentration of the compound in the environment.

BACKGROUND

EPA is sometimes taken to task for running a pesticide registration process that is unbending and unilateral. The registration of pheromones and other biological pesticides belies that charge. The Agency's strategy for registering biological pesticides exemplifies foresight and flexibility. Since the pheromone of the female silkworm moth was isolated and identified by Butenandt and his associates in 1959, this class of compounds was recognized as a potentially safer method of controlling damaging populations of economically important insect species. Anticipating the future importance of pheromones and other biochemicals, in May of 1974 the EPA's Office of Pesticide Programs sponsored the development of a report which was to contain scientific data and other technical information specific to these compounds. This report was the foundation document which enabled the Agency to develop separate registration guidelines to direct the development and registration of pheromones and insect growth regulators. Within the context of the report, the term "pheromone" included all biochemicals able to modify pest behavior and encompassed all naturally occurring semiochemicals.

In 1979, the Agency published a policy statement in the Federal Register which encouraged the development and registration of pheromones and similar biochemicals considered to be potentially safer alternatives to conventional pesticide products. In November of 1982, the Agency published the EPA Assessment Guidelines for Microbial and Biochemical Pesticides, Subdivision M. The target time for review of an application for a new registration of a biological pesticide is 280 days, or about half that for a conventional application. However, this target can be met only if the application is complete. As a result of these initial efforts, EPA has registered 41 biochemical products and 21 microorganism products.

REGISTRATION REQUIREMENTS

The Subdivision M Guidelines and the companion regulations in 40 CFR, Section 158.65 significantly altered the data requirements for microbial and biochemical pest control agents. The result is considerably reduced data submission requirements for biological products when compared to the typical pesticide product. The decreased data requirements are predicated upon the nature of these substances, which is inherently different from conventional pesticides. Biological pesticides are characterized by their relatively low toxicities and limited exposure potential, target species specificity, and natural occurrence. Typically, their formulations and delivery methods offer low exposure potential and application is made at low rates--commonly 20 grams per acre or less--to terrestrial sites. Additionally, these are highly volatile compounds.

The Agency devised a "tier testing scheme" for the major data categories: product chemistry, toxicology, residue chemistry, nontarget organism hazard, and environmental fate and effects. When the Agency determines that a product's Tier I data is satisfactory, that no significant adverse effects have been demonstrated, then no additional testing is required.

Product Chemistry

These data are always required so that the Agency can know what the product is. The data requirements for product analysis include identification both of the product and the manufacturing process. The chemical structure of each active ingredient should be provided, as well as a discussion of any unintentional ingredients. An analysis of samples may be conditionally required. The data submission should include a certification of limits, description of analytical methodology and listing of the product's physical and chemical properties. The submission of samples is encouraged, and may be conditionally required.

Toxicological Data

These data requirements are imposed on a case-by-case basis, using a three-tier approach. The uses proposed for a pesticide product and the risks inherent in those uses determine the applicable requirements. The first tier of tests addresses the Agency's major concern with the use of pheromones --acute toxicity, primary eye and dermal irritation, hypersensitivity, mutagenicity/genotoxicity and cellular immune response. Upon submission of adequate scientific rationale, some Tier I toxicological data requirements are frequently waived. If the Tier I toxicological data show significant adverse effects, additional Tier II or III data may be required.

Should a tolerance be sought or required, the Agency will seek data on developmental effects and a 90-day feeding for a feed/food use. However, these data requirements are often waived if the applicant product is identical to that occurring in nature, and if the results of the mutagenicity/genotoxicity and acute toxicological data series were acceptable. All currently registered food-use pheromones are exempt from tolerance requirements based on acceptable first tier testing.

Residue Chemistry

Residue chemistry data may be required for one of two reasons. First, whenever a biological pesticide is to be applied on feed or food crops at a rate of greater than 20 grams active ingredient per acre per application, the Agency must either establish a tolerance specifying the amount of allowable residue or issue an exemption from the tolerance requirement. Standard residue chemistry data identifying the nature and magnitude of residues likely to occur are necessary in this case. Should no residues be identified, a rationale should accompany this disclosure. Second, similar residue data are required when a review of Tier I toxicological data yields unsatisfactory results.

Registrants may also provide the Agency with a convincing scientific rationale to support a contention that no residues will occur as the result of use of the pesticide. Should the Agency accept the rationale, the requirement for residue chemistry data can be waived. Data describing background levels from pest infestations are extremely helpful in facilitating the Agency's decision-making on this point.

Non-target Organism Hazard and Environmental Fate and Effect Data

These data requirements, like the toxicology data requirements, are tiered. Their purpose is to identify the risks that the product, when used as intended, will pose to non-target organisms. The Tier I testing is concerned with four areas of potential hazard: terrestrial wildlife; aquatic animals; plants and beneficial insects. Typically, the Agency will require the standard Tier I, avian, fish and aquatic invertebrate toxicity studies. Some Tier I data requirements may be made conditional if the application involves impregnation of the pheromone in a matrix or the use pattern presents little opportunity for significant exposure to occur. Again, effects determined at the first tier will dictate additional testing. Testing prescribed in Tier II seeks to identify environmental effects while Tier III testing both evaluates the nature of the risk posed and quantifies the extent of the potential hazard.

Experimental Use Permits

Inert ingredients of all pesticide products, including pheromones, are evaluated by EPA and also must receive a clearance under the Federal Food, Drug and Cosmetic Act (FFDCA) if the pheromone product is for a food use. If the inert has not been evaluated previously or received a clearance, then the applicant must submit appropriate data on the inert ingredient and allow time for the evaluation and clearance process.

FIFRA does not require registrants to obtain an experimental use permit (EUP) in order to conduct small-scale (10 acres or less) field tests. Generally, test sites of greater than ten acres will require an EUP.

The permit requirements are based on the reduced data requirements applicable to biologicals. Where food crops are involved and will not be destroyed at the conclusion of testing, a temporary tolerance or an exemption from that requirement must be approved prior to the Agency issuing an EUP. Generic EUPs are issued when a registrant wishes to test more than one formulation of a product, as long as there is no change in the active ingredient. Additionally, varying parameters for product testing (e.g., multiple year testing and a range of release rates of chemical in a dispenser) under a single EUP are usually approved.

Data Waivers

The Agency realized that the regulations and Subdivision M Guidelines were necessarily general and that some of the data prescribed might not apply to or be feasible for all active ingredients, formulations and/or patterns of use. With this in mind, a provision was included in the regulations which permitted a potential applicant to develop a scientific justification to support a request to waive data requirements not believed to be relevant to the evaluation of the hazard presented by the product under consideration. It is the registrant's responsibility to initiate the request for a waiver and to provide a scientific rationale to substantiate each request.

AGENCY APPROACH TO THE REGISTRATION PROCESS

It should be recognized that the Subdivision M Guidelines and the companion regulations in 40 CFR, Section 158.65 apply to a broad group of biochemicals. That they may all occur naturally in the environment does not necessarily mean that they are free of hazards when utilized by humans. Some of the most toxic substances known occur in nature.

The tier testing scheme subjects the chemicals to a maximum hazard challenge in terms of dose, concentration or route of administration in testing for human hazard and non-target organism risk assessment. Because the tiered scheme ensures that only the minimum data set is required, it works favorably for the registrant. Yet it permits the identification of any hazards associated with the use of the product, providing the Agency with the sound scientific basis required for a regulatory decision. If, in an initial tier of studies the results demonstrated that the compounds exhibited no significant adverse effects, then no further testing would be required. Detection of adverse effects in the first tier compel completion of the second and perhaps third tiers of tests to evaluate and quantify the actual hazard posed. In summary, the Agency has developed a base set of data requirements which are routinely required of pheromones: product chemistry data, certain Tier I mammalian tox data and certain Tier I ecotox data. In the cases of both Tier I mammalian and ecotox data, the Agency has in some cases either waived requirements altogether or granted registrations on the condition that the additional Tier I data be developed and submitted to the Agency. To date, for pheromones, the Agency typically has not required residue chemistry data or the advanced Tier toxicology and ecological effects and environmental fate data. No significant effects have been demonstrated in the first tier maximum hazard challenge and so no additional studies have been required.

The Agency has received and has routinely acted favorably upon requests to waive data requirements for pheromone registrations. To date, however, EPA has resisted requests to generically waive data requirements for the entire class of compounds. Approval of such a generic waiver could only be based on the results of extensive analysis of the available data in order to assure that EPA's responsibility to protect the public from unreasonable risk has not been compromised. The Agency's processes are viewed by many as reasonable regulatory action. In 1987, Dr. Albert Minks from the Research Institute for Plant Protection in Wageningen, The Netherlands, observed that the U.S. Guidelines for pheromone registration should be used as the basis for the development of similar rules in other countries. Several years after the official publication of the EPA Guidelines for Microbial and Biochemical Pesticides, it can be concluded that these Guidelines and the Agency's application of them in specific cases have provided both substantial flexibility and the necessary scientific basis for regulating biologicals.

Nevertheless, there are pleas from the scientific and academic communities as well as the regulated industry for more flexibility, exemptions and speed in the registration process. Such pleas are convincing testimony that the registration process for biologicals is still in its youth. From the Agency's internal perspective, the process is already creative, sensible and yet scientifically sound. Biological applications are given priority, consistent with competing priorities in

terms of times and resources. This priority ranking applies from front-end processing to the workings of the Biotechnical Work Group to the final decision process.

The work group is comprised of professionals who espouse a special interest in the registration of biological products. This group not only evaluates the individual applications we receive but also is reexamining the regulatory process as well as the Agency's internal registration procedures. The group is attempting to identify both perceived and real impediments to registration and is looking for ways to further amend or streamline the system and processes. This is being accomplished in part through the development of an OPP "Biologicals Work Plan." This Plan will set forth the results of the work group's systematic review of our current program and will recommend to Agency management changes for improving it. Such changes may include the possibility of generic EUPs and additional opportunities for exemptions from certain data requirements.

But in the interim, know that successful registration applications are the result of good-faith efforts at compliance with the requirements currently in-place. Specifically, schedule pre-application conferences with the appropriate product manager to determine what data are needed in the submission. Prepare a summary of the decisions and recommendations resulting from that conference, and request that EPA confirm your interpretation of pertinent requirements.

Exercise quality control over all data and submissions--and use the Agency's mail codes to speed your application to its destination. Contact EPA to identify problems early and to track the submission's progress, but maintain a realistic sense of processing time. Recognize that success within the current system and progress in refining the registration process for biological pesticides are both dependent upon a solid partnership.

PROGRESS AND PARTNERSHIP

Of the many constraints under which the Agency operates, perhaps the most significant is the need to base all regulatory decisions upon sound scientific evidence. EPA can make decisions when appropriate and adequate data confirms that the actions proposed both safeguard our populace and are in the public interest.

With this in mind, the Office of Pesticide Programs has undertaken the development of a paper which attempts to assemble all relevant data and technical information concerning the chemical, physical and toxicological properties of a class of pheromones and related semiochemicals, namely the straight chain primary alcohols, aldehydes and acetate esters of straight chain primary alcohols. An early draft of that paper

is just now entering internal peer review within the Office of Pesticide Programs. It is expected that analysis of the relevant data for this class of pheromone and related semiochemicals (i.e., certain plant volatiles) will permit the agency to further adjust our data requirement for this specific class of biochemicals. This structural analysis will serve as the scientific basis for regulatory relief.

It is the Agency's intention to have the draft paper also reflect the comments of working scientists external to EPA. It is hoped that when finalized in the summer of 1991, the paper will serve as a foundation for future amendments to regulations and secure EPA's place as leader for regulatory relief for pheromones.

While the straight chain alcohols, aldehydes and acetates comprise the largest single group of known semiochemicals, many other diverse classes of compounds are also involved. If EPA is indeed going to meet the expectations of those involved in developing biologicals, the Agency needs to start now to develop a more efficient process for developing similar structural analyses of the other classes of pheromones and similar semiochemicals. The draft paper on straight chain alcohols, aldehydes and acetates has, as you know, been under development for a long time. While the paper uses relevant data submitted to the Agency in a limited number of petitions, among other sources, the bulk of the work has been in gathering, organizing and analyzing data which has been widely available. In this case, EPA staff has done this work though many others could have. In fact, EPA probably would not have done this work at all for conventional chemical class of compounds, but rather, would have expected the producing industry to take the lead. We were willing to do the work in this case because of the inherent promise of pheromones to provide safer pest control. But if we are to make greater, more rapid strides in the future, it would be to our mutual benefit to work together to bring the biological registration process to maturation. Much more could be accomplished if EPA, the scientific and academic communities and the regulated industry shared resources and ingenuity to form a consortium dedicated to developing a biological registration strategy. Such a consortium would possess both the expertise and desire to identify and scientifically justify alternatives to those issues currently perceived as impediments to registration. A consortium may indeed be the mechanism needed to advance the objectives common to all. I urge you to seize the opportunity to become a part of the solution you seek.

CURRENT AND PROPOSED REGULATIONS.

STATUS IN EUROPE.

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ABSTRACT

The status of pesticide regulations in Europe is reviewed in this paper with reference to pheromones and other semiochemicals. With the EC draft Directive on pesticide registration shortly to be adopted by the Community, its effect on the registration procedures for pheromones is discussed. Most European countries do not require pheromones which are used in traps for population monitoring to be registered but if they are used to control populations then they are regarded as insecticides both by EEC member countries and by the remaining countries of Europe. Most countries however recognise the inherent benign nature of these chemicals and the low levels of risk that they present and for this reason have demonstrated a significant degree of flexibility in terms of the data which are required for their registration. The opportunity now exists with the EC Directive to harmonize their registration requirements within the EEC.

INTRODUCTION

Over the last 30 years, most European countries have developed comprehensive mechanisms and procedures for registering pesticides. In many cases, however, the data requirements have differed quite extensively from country to country. With the background of the 1992 Single European Act, the European Commission has issued a proposed EC Directive to harmonize the registration requirements between the various member countries (European Commission, 1989). The Directive was thoroughly reviewed and discussed at a British Crop Protection Council Symposium held in Reading, UK, in January 1990 and the proceedings of that meeting (Thomas, 1990) serve as a good reference work in understanding the proposed harmonisation of registration procedures.

During the Reading Symposium, the registration requirements under the new Directive for semiochemicals or other biorational pest control products were not discussed and it was hoped that this question could have been addressed directly by a representative of the European Commission at the current Symposium, the proceedings of which form the basis of this Monograph. Unfortunately, due to unavoidable circumstances, no representative of the EC could attend this Symposium and give the EC view on the registration requirements of pheromones and other semiochemicals. However, a representative of the Directorate General for Agriculture (DG VI) was prepared to grant Mme N. Verbiese, a contributor to this volume, an interview on this subject prior to the Symposium and the comments stated below relating to the EC view come predominantly from the views expressed by the EC representative at that meeting.

The European Economic Community, of course, does not represent the whole of Europe and the question of semiochemical registration applies equally to both EEC and non-EEC countries alike. In this context, the paper presented by Minks (1990) at the 1987 Entomological Society of

America Symposium in Boston gave an accurate evaluation of the registration requirements for pheromones in both EEC and non EEC countries in Europe. Some of the progress that has occurred since that paper was written will be mentioned here but for a full historical perspective of pheromones, their use and registration in Europe, reference should be made to the above mentioned paper (Minks, 1990).

THE PROPOSED EC DIRECTIVE.

At the time of writing this paper, the above Directive has not yet been adopted by the European Parliament and no mechanism exists currently, therefore, for registering plant protection chemicals at the EC level. This task is presently carried out within the member countries only and their role in the proposed Directive is detailed by Verbiess (this volume). Within the new Directive, pheromones or other semiochemicals are not regarded as a separate group of compounds and as the Directive stands, they will be regarded as insecticides if their intended use is to control insect populations. The EC, however, recognises that semiochemicals control insect populations through benign means which do not involve direct toxic effects on the target species, and as such, may be viewed differently in terms of the data required for their registration. The possibility of any data waivers, however, would depend on the EC receiving sound scientific evidence upon which decisions to simplify the process could be made.

The EC would welcome a unified approach to the simplification procedure for semiochemical registration. They would expect there to be mutual data recognition between member countries of the EEC and they welcome a dialogue with all interested parties with regard to the registration of semiochemicals.

Whereas it is unlikely that specific mention of semiochemicals will be made in the Directive, there is scope for discussing the registration of pheromones and other semiochemicals as a separate issue before the implementation stage of the Directive begins in January 1993.

THE CURRENT SITUATION REGARDING THE REGISTRATION OF PHEROMONE OR OTHER SEMIOCHEMICAL-BASED PRODUCTS WITHIN MEMBER COUNTRIES OF THE EEC.

If a pheromone is used in a trap for detection or monitoring of a pest population, then registration is normally not required in any country. However, there may be a requirement to notify the appropriate authorities that a particular monitoring system is to be marketed in a particular country and there may be other regulations which have to be observed during the manufacture of such systems such as the Control of Substances Hazardous to Health regulations in the UK. However, because of the very small amounts of pheromone used in such systems and their very benign nature, such legislation has not inhibited their development as monitoring tools in pest management.

In most EEC member countries, however, if a pheromone product is to be used in controlling pest populations then some measure of registration of the product is required. In the Addendum to Minks(1990) paper, there is a detailed survey of 14 European countries regarding the status of pheromone registration and over the past three years since that paper was presented at Boston, not much has changed in terms of procedures or numbers of pheromone based products registered. The following points would summarise the situation within the member countries of the EEC as at December 1990:

- Countries where semiochemical-based products have been registered include France and Germany. In France, a mass trapping product for Ips typographus and a mating disruption formulation for Grapholitha molesta have been registered, while in Germany, a mating disruption formulation for Eupoecilia ambiguella has been registered.

- Countries where submissions have been made for registration of pheromone-based products but no approvals have been made to date include Spain and the Netherlands. In Spain, although full approval has not been granted for the commercial use of pheromone based products, projects supervised by the Ministry of Agriculture, or their equivalent in the Autonomous regions of Spain, have been permitted for mating disruption of certain vine, peach and rice pests. In the Netherlands, on the other hand, no approval of a pheromone based product has been granted despite the fact that one application in particular for registration of an aphid alarm pheromone has been under review by the Dutch Bureau of Pesticides for over five years.

None of the EEC member countries have special procedures or regulations such as those of the Environmental Protection Agency in the U.S.A. (Lindsay, this volume) for registration of pheromones or biorational products. On the contrary, in every country where the question has arisen, pheromones and other semiochemicals used for insect control are regarded as insecticides and are subject to the same rules and regulation governing their registration. It has to be said, however, that in the majority of countries within the EEC where pheromone-based products have, or are being, registered, the registration authorities have demonstrated a considerable degree of flexibility with regard to the data requirements, recognising the benign nature of the compounds concerned and the low levels of risk involved in their use. Factors which have been taken into account when deciding on the degree to which data requirements can be waived include the dosage of pheromone per hectare, the form of application, i.e. hand applied versus sprayable formulations, and the degree of exposure to the applicator, the consumer and the environment. Most member countries have welcomed the experimental use of pheromone-based products within their territories and have not placed undue restrictions on their field evaluation. Moreover, the Ministries of Agriculture, in their various forms, in the majority of member countries have been actively involved in the development of pheromone-based insect monitoring and control technology.

THE CURRENT SITUATION REGARDING THE REGISTRATION OF PHEROMONE OR OTHER SEMIOCHEMICAL-BASED PRODUCTS IN EUROPEAN COUNTRIES OUTSIDE THE EEC.

As in the case of the EEC countries, if a pheromone is used for monitoring insect populations, it does not have to be registered in any country in Europe outside the EEC other than in Czechoslovakia where tests of efficacy and quality of trapping systems are obligatory before registration is granted.

Austria, Norway and Switzerland all have products based on pheromones which have been registered for insect pest control. In Austria, products for mating disruption of both Cydia pomonella and Eupoecilia ambiguella have been registered while in Norway, an extensive mass trapping campaign against Ips typographus was permitted during the years 1979 to 1983. In Switzerland, provisional authorization has been granted for mating disruption products aimed at Cydia pomonella and Eupoecilia ambiguella.

In the remainder of the Western European countries and in Eastern Europe, it is known that significant activity is taking place regarding the field evaluation of pheromone-based products for control of insect pests of agriculture and forestry but no specific registrations are known to the author at present.

CONCLUSIONS.

In conclusion therefore, most European countries have taken a similar stance regarding the registration of pheromone-based products. When pheromones are used in traps for detection and monitoring, no registration has been required in any country other than Czechoslovakia and the use of pheromones for this purpose is well established throughout Europe.

If the intention is to use pheromones or other semiochemicals for population suppression (control as opposed to monitoring) then the product needs to be registered as an insecticide. Flexibility has been shown, however, by most European countries and data waivers have been granted in most cases especially when sound scientific evidence has been presented to support a request for such waivers. The EC is attempting to harmonize pesticide regulation within the Community and is very open to dialogue on the subject of semiochemicals and their registration. The opportunity clearly exists for an unified approach to the way pheromones and other semiochemicals are handled by the registration authorities of the EC and its member countries as well as the rest of Europe. The development of pheromone and other semiochemical based products as part of an integrated pest management strategy can be expedited through the regulatory process provided sound scientific evidence is made available to the regulatory authorities which supports the arguments for them being treated with flexibility and calculated leniency.

ACKNOWLEDGEMENTS.

I am indebted to Nicole Verbiese for relaying to me the outcome of her discussions with EC representatives on the subject of semiochemical registration.

REFERENCES.

- European Commission (1989). Amended proposal for a Council Directive concerning the placing of EEC-accepted plant protection products on the market. Official Journal No C 89, 10 April, 1989, p22. COM (89) 34 final.
- Minks, A.K. (1990). Registration requirements and status for pheromones in Europe and other countries. In: Behaviour-Modifying Chemicals for Insect Management. Ridgway, R.L., Silverstein, R.M. and Inscoc, M.N., (Eds), Marcel Dekker Inc., New York and Basel. pp 557-568.
- Thomas, B. (1990). Future Changes in Pesticide Registration within the EC. Monograph No. 44 - Proceedings of a British Crop Protection Council Symposium held at Reading, UK, 3 - 5th January, 1990. pp121.

4. Non-Target Effects

Chairman: HEINRICH ARN

NON-TARGET EFFECTS OF LEPIDOPTERAN SEX ATTRACTANT PHEROMONES

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ABSTRACT

In assessing the suitability of a substance for use in pest control, the effects of the material on organisms other than the target pest must be considered. Information on non-target effects of compounds that are known to be pheromones of lepidopteran pests or are closely related in chemical structure is reviewed. Data on mammalian toxicity, fish and avian toxicity, effects on nontarget insect species, anticipated exposures, and environmental fate suggest that there is minimal risk associated with the use of sex attractant pheromones produced by lepidopteran females.

INTRODUCTION

In evaluating the potential risk associated with the use of a substance for controlling an insect pest, the various ways in which species other than the target species might be affected must be considered. A knowledge of mammalian toxicity is important, since clearly the possible effects of the substance on workers handling the material or on humans or other mammals that might come into contact with the material need to be understood. Likewise, possible effects on birds or on aquatic organisms of any material released into the environment must be evaluated. It is also desirable to examine the effects the material might have on insects other than the target pest. Finally, since the hazard associated with the use of a substance will be a function both of its inherent toxicity and of anticipated exposures, the amount to be used and its fate in the environment must also be taken into consideration. The kinds of data that are needed to assist in assessing risk in registering pheromones and other semiochemicals in the United States have recently been discussed within the context of the tier-testing system used for biochemical insecticides by the US Environmental Protection Agency (EPA) (Tinsworth 1990; Lindsay, this volume).

LEPIDOPTERAN SEX ATTRACTANT PHEROMONES

Insects use many types of pheromones for intraspecific communication; some of the most familiar are sex pheromones, aggregation pheromones, alarm pheromones, or oviposition deterrent pheromones. The term "pheromone" is often used without any qualification to refer to sex attractant pheromones or aggregation pheromones that can be used to lure insects into traps or interfere with their mating communication, but this should only be done after defining the specific type of pheromone involved.

It has frequently been stated that sex and aggregation pheromones, especially lepidopteran pheromones, are relatively nontoxic and are used in such low doses that they pose essentially no environmental or health hazards when used for pest management. The validity of such a statement will be discussed here in light of data in the published literature or other reports that were available to the authors. Undoubtedly, additional data on pheromones that have been registered exist in the files of the registrants and of the regulatory agencies, but these are not readily available to the public.

For lepidopteran species using sex attractant pheromones, it is usually the female that emits the pheromone to attract males. Most female lepidopteran sex pheromones that have been identified:

- (1) are straight-chain compounds with 10 to 20 carbon atoms in the chain,
- (2) have no unsaturation or have 1, 2, or 3 double bonds,
- (3) are alcohols, acetates, or aldehydes, and
- (4) show little or no toxicity.

A survey of commercial suppliers showed that synthetic pheromones for monitoring were offered for about 250 insects, of which about 200 were Lepidoptera (Inscoc *et al.* 1990). A tally of the components of these commercially offered lepidopteran pheromones is given in Table 1. A compound was counted each time it was reported as a pheromone component, and no distinction was made between geometric [(Z) and (E)] isomers. Many of these insects use mixtures of several components as pheromones, so the total number of components represented here is about 350. Only 15 of the components represented in Table 1 are not alcohols, acetates or aldehydes; these 15 include 7 hydrocarbons with 17-21 carbon atoms, 5 ketones with 19-21 carbon atoms, 2 branched-chain epoxides with 19 carbon atoms, and two branched-chain esters of straight-chain acids with 10 or 14 carbon atoms. The great majority of the pheromone components involved are straight-chain compounds with 10 to 18 carbon atoms in the chain; and they are saturated or have 1, 2, or 3 double bonds. These data clearly bear out the first three generalizations mentioned above. The fourth generalization, on the relative non-toxicity of these pheromones, will be discussed later.

TABLE 1. Components of commercially available pheromones for 200 lepidopteran species (From Inscoc *et al.* 1990).

Components ¹	Numbers of carbon atoms in chain									
	9	10	11	12	13	14	16	17	18	>18
Acetates	—	7	—	65	2	117	29	—	12	—
Alcohols	—	2	—	10	—	31	15	—	4	—
Aldehydes	1	—	2	1	2	11	34	—	5	—
Miscellaneous ²	—	1	—	—	—	1	—	2	2	9

¹ Geometric isomers were not counted separately. A compound was counted each time it was reported.

² 4 ketones (C₁₉₋₂₁), 7 hydrocarbons (C₁₇₋₂₁), 2 branched-chain epoxides (C₁₈), and 2 branched-chain esters of long-chain acids.

Components of lepidopteran pheromone products that have been registered by the EPA show a similar trend (Table 2). Of 10 esters in registered formulations, 9 are acetates of unsaturated long-chain alcohols; the 2 alcohols and 6 aldehydes also fit the generalization. The only registered compounds not fitting the general structure are the pheromone of the bagworm (*Thyridopterix ephemeraeformis*), a branched-chain ester of decanoic acid, and disparlure, the pheromone of the gypsy moth (*Lymantria dispar*), an oxirane [epoxide].

TABLE 2. Lepidopteran pheromone components in products that have been registered by US EPA (From Tinsworth 1990).

ESTERS

(Z)-8-Dodecen-1-ol acetate
(E)-8-Dodecen-1-ol acetate
(Z)-9-Dodecen-1-ol acetate
(E)-9-Dodecen-1-ol acetate
(Z)-4-Tridecen-1-ol acetate
(E)-4-Tridecen-1-ol acetate
(Z,Z)-7,11-Hexadecadien-1-ol acetate
(Z,E)-7,11-Hexadecadien-1-ol acetate
(E,Z)-3,13-Octadecadien-1-ol acetate
(Z,Z)-3,13-Octadecadien-1-ol acetate

(R)-1-Methylbutyl decanoate

ALCOHOLS

(Z)-8-Dodecen-1-ol
(Z)-11-Hexadecen-1-ol

ALDEHYDES

Tetradecanal
(Z)-9-Tetradecenal
Hexadecanal
(Z)-7-Hexadecenal
(Z)-9-Hexadecenal
(Z)-11-Hexadecenal

OTHER

cis-2-Decyl-3-(5-methylhexyl)oxirane

With few exceptions, then, the known lepidopteran sex attractant pheromones represent a well-defined group of compounds made up of 3 homologous series, each having a polar group at one end of a straight chain of carbon atoms. In general, many of the properties of compounds in such a homologous aliphatic series change fairly predictably as the carbon chain is lengthened, as demonstrated with a series of alcohols (Table 3). Many of the alcohols shown in this table are used in cosmetics or as pharmaceutical aids, and some are lepidopteran pheromone components. The melting points and boiling points of these compounds increase with the lengthening chain, while the solubility in water decreases. This decrease in aqueous solubility appears to have some relation to the observation discussed by Albert (1968) in his classic book on "Selective Toxicity", in which, above a critical chain length, lengthening of the carbon chain of primary alcohols is associated with a decrease in toxicity toward organisms such as the bacterium, *Staphylococcus aureus*.

The last two alcohols in Table 3, octadecanol (stearyl alcohol) and (Z)-9-octadecenol (oleyl alcohol), illustrate effects the introduction of a double bond may have on the physical properties of a compound. Octadecanol occurs at room temperature as slippery flakes or granules melting near 60°C. The (Z)-9- double bond introduces a kink or rigid portion into the middle of the hydrocarbon chain that makes it more difficult for the molecules to become aligned in changing from the liquid to the solid phase; consequently the melting point is lower, 13°-19°C. Since the double bond has little effect on the ease with which a molecule passes from a liquid to a vapor state, there is less of a difference between the boiling points of the two compounds.

TABLE 3. Properties of aliphatic alcohols (From *Merck Index* 1983).

Alcohol	Melting Point (°C)	Boiling Point (°C)	Properties	Uses
1-Octanol (caprylic alcohol)	-16° to -17°	194-195° (760mm Hg)	Colorless liquid	Manuf. of perfumes, esters
1-Decanol (<i>n</i> -decyl alcohol)	6.4°	233° (760mm Hg) 109.5 (8mm Hg)	Viscous liquid	Manuf. of solvents, surface-active agents, herbicides, plasticizers
1-Dodecanol (lauryl alcohol)	24°	259° (760mm Hg) 134.7° (10mm Hg)	Leaflets	Manuf. of wetting agents
1-Tetradecanol (myristyl alcohol)	38°	167° (15 mm Hg)	White crystals	Emollient in cold creams, manuf. of wetting agent for textiles
1-Hexadecanol (cetyl alcohol)	49°	344° (760mm Hg) 190° (15mm Hg)	White crystals	Cosmetics (emollient, emulsion modifier), pharmaceutical aid (emulsifying, stiffening agent)
1-Octadecanol (stearyl alcohol)	59.4-59.8°	210° (15 mm Hg)	Unctuous white flakes, granules	Cosmetics, pharmaceutical aid, emulsifier, antifoam agent
(<i>Z</i>)-9-Octadecen-1-ol (oleyl alcohol)	13-19°	195° (8mm Hg)	Oily liquid	Manuf. of detergents, antifoam agent, carrier for medicaments

TABLE 4. Mammalian toxicity of some components of lepidopteran pheromones.

Compound	Acute oral LD ₅₀ (rats, mg/kg)	Acute dermal LD ₅₀ (rabbits, mg/kg)	Eye irritation (rabbit ¹)	Skin irritation (rabbits ²)	Inhalation (rats, mg/l ³)	Mutagenicity (Ames assay)
<u>Alcohols</u>						
(Z)-7-Dodecenol	≥11,730 ⁴	~3700 ⁴	10.1 (24 hr) ⁴	8.0 ⁴	>6.7 ⁴	—
(E,E)-8,10-Dodecadienol	>3,250 ⁵	not irritant ⁵	not irritant ⁵	not irritant ⁵	—	—
(Z)-11-Hexadecenol	>5,000 ⁶	—	—	—	—	—
<u>Acetates</u>						
(Z)-7-Dodecenol acetate	≥13,430 ⁴	>2025 ⁴	0 (24 hr) ⁴	2.8 ⁴	>4.5 ⁴	—
(Z+E)-8-Dodecenol acetate	>17,100 ⁷	>20,000 ⁷	not irritant ⁷	sl. irritant ⁷	>74.7 ⁷	not mutagenic ⁷
(Z+E)-9-Dodecenol acetate	>15,000 ⁸	>3,000 ⁸	sl. irritant ⁸	not irritant ⁸	not toxic ⁸	—
(E)-4-Tridecenol acetate	no mortalities, 24 hr ⁷	no mortalities, 13 d ⁷	not irritant ⁷	sl. irritant ⁷	not toxic ⁷	not mutagenic ⁷
(Z)-9-Tetradecenol acetate	>5,000 ⁶	—	—	—	—	—
(Z)-7-Hexadecenol acetate	>34,600 ⁴	>2025 ⁴	1.3 (24 hr) ⁴	1.9 ⁴	>3.8 ⁴	—
(ZZ+ZE)-7,11-Hexadecadienol acetate	>15,000 ⁸	—	not irritant ⁸	not irritant ⁸	>3.3 ⁸	not mutagenic ⁷
(ZZ)-3,13-Octadecadienol acetate	>5,000 ⁶	—	—	—	—	—
(EZ+ZZ)-3,13-Octadecadienol acetate	nae ^{7,9}	nae ⁷	nae ⁷	nae ⁷	nae ⁷	—
<u>Aldehydes</u>						
Tetradecanal	>5,000 ⁶	—	—	—	—	—
(Z)-9-Tetradecenal	>5,000 ⁶	—	—	—	>5 ¹⁰	—
(E+Z)-11-Tetradecenal	>5,000 ^{7,8}	>5,000 ^{7,8}	not irritant ^{7,8}	slight to moderate irritant ⁸	>16.88 ^{7,8}	—
Hexadecanal	>5,000 ⁶	—	—	—	—	—
(Z)-7-Hexadecenal	>5,000 ⁶	—	—	—	—	—
(Z)-9-Hexadecenal	>5,000 ⁶	—	—	—	—	—
(Z)-11-Hexadecenal	>5,000 ⁶	—	—	—	>5 ¹¹	—

¹ Draize scoring system. Maximum score is 110. ² Draize scoring system. Maximum score is 8.0. ³ No-effect level. ⁴ Beroza *et al.* 1975. ⁵ Knipling 1976. ⁶ Teeters 1979. ⁷ Kirsch 1988. ⁸ Hodosh *et al.* 1985. ⁹ nae=no apparent effect. ¹⁰ Cannon Laboratories 1980. ¹¹ Cannon Laboratories 1979.

MAMMALIAN TOXICITY

Published mammalian toxicological data on pheromone components are somewhat limited, but data on three alcohols, nine acetates and seven aldehydes are presented in Table 4. In general, the acute oral, acute dermal, eye and skin irritation, inhalation, and mutagenicity tests with lepidopteran pheromone components indicated that these compounds have relatively low toxicities. The oral LD₅₀s in rats listed for the compounds tested were all greater than 3,000 mg/kg, and since these values were generally reflect results of "limit testing", the actual values may be much greater. Other than skin irritation caused by one of the alcohols, no significant adverse effects were reported for these materials. To put these data in perspective, oral LD₅₀s for five of these compounds are compared in Table 5 with those for some other naturally occurring compounds and some common insecticides.

TABLE 5. Representative toxicities of selected naturally occurring compounds and pesticides.

Compound	LD ₅₀ in rats (mg/kg)	Compound	LD ₅₀ in rats (mg/kg)
Parathion-methyl ¹	14-24	(<i>E,E</i>)-8,10-Dodecenol ³	>3,250
Nicotine ¹	50-60	(<i>E + Z</i>)-11-Tetradecenal ⁴	>5,000
Dichlorvos ¹	56-108	(<i>Z</i>)-7-Dodecenol acetate ⁵	≥13,430
<i>p</i> -Cresol ²	207	(<i>Z+E</i>)-9-Dodecenol acetate ⁶	>15,000
<i>m</i> -Cresol ²	242	(<i>ZZ+ZE</i>)-7,11-Hexadecen- ol acetate ⁶	>15,000
Pyrethrins ¹	584-900		
Linalool ²	2,790		
Malathion ¹	2,800		

¹ Worthing 1979. ² US National Institute of Occupational Safety and Health 1982. ³ Knipling 1976.

⁴ Kirsch 1988. ⁵ Beroza *et al.* 1975. ⁶Hodosh *et al.* 1985.

Daughtrey *et al.* (1989a,b; 1990) have reported studies on the teratogenic potential of commercial "octyl acetate" and the subchronic toxicity of commercial "octyl" and "tridecyl acetates" in rats. Despite their names, these materials are acetates of branched-chain alcohols and are not strictly homologues of the pheromone acetates being considered here. Nevertheless, the properties of these compounds would be expected to be similar. The data indicate that "octyl acetate" "is not a selective developmental toxicant" and that both esters are "of a low order of toxicity" following subchronic administration at doses up to 1 g/kg to rats. These authors also state that as a general class, the saturated aliphatic acetates are characterized as having low toxicity.

AVIAN AND FISH TOXICITY

Available information on dietary toxicity studies on birds (quail and duck) and fish (rainbow trout and bluegill) with some pheromone components is summarized in Table 6. Again, no significant adverse effects were reported with one acetate and two acetate mixtures. (*Z*)-7-Dodecen-1-ol was slightly toxic to the two fish species.

NON-TARGET INSECTS

Early workers in the pheromone field thought that each insect used a single characteristic compound as its attractant pheromone. Within a very few years, however, it was found that

TABLE 6. Non-mammalian toxicity of lepidopteran pheromone components.

Compound	8-day dietary LC ₅₀ (ppm)		96-hr static LC ₅₀ (ppm)	
	Quail	Duck	Rainbow trout	Bluegill
(Z)-7-Dodecenol	—	—	3.7 ¹	2.8 ¹
(Z)-7-Dodecenol acetate	—	—	>100 ¹	>100 ¹
(Z+E)-9-Docecenol acetate	>5,000 ²	>22, ³	—	>300 ²
(ZZ+ZE)-7,11-Hexadeca-dienol acetate	>10,000 ²	>102, ³	270 ²	540 ²

¹ Beroza *et al.* 1975. ² Hodosh *et al.* 1985. ³ g/kg body weight.

many insects used blends of compounds, and that a given compound could be a component in the pheromone blends of several insects. For example, by 1980, (Z)-11-tetradecenyl acetate had been identified as the pheromone of or a component in the pheromone blend of 18 insect species (Inscoc 1982). Specificity of pheromone response between different lepidopteran insect species using the same pheromone component can be achieved by the presence of different components in the pheromone blends, different ratios of components, geographic differences in habitat, or variations in circadian rhythms. In order to achieve the desired specificity of trap catch in monitoring traps, it is sometimes necessary to include additional compounds in a lure or to adjust the component ratios in order to reduce the attractancy of the lure to another species. However, such lack of pheromone specificity in monitoring would not be expected to lead to problems or hazards in insect control programs.

An insect pheromone may also act as a kairomone, attracting parasites or predators. For example, some predatory insects (*Thanasimus* spp.) are attracted by *cis*-verbenol and ipsdienol, components of the *Ips typographus* pheromone. In a mass trapping project against *I. typographus* in Norway, it was necessary to make the holes in the trap tubes small enough to exclude these beneficial predators (Bakke & Lie 1989) so as to maintain the population of these natural enemies. Attraction of parasites and predators is not expected to be a problem in control tactics like mating disruption of Lepidoptera, since the parasites and predators belong to different orders than their prey and their sex pheromones would have quite different chemical structures. For example, components of the sex pheromone of the pentatomid predator *Podisus maculiventris* are α -terpineol and (*E*)-2-hexenal (Aldrich *et al.* 1984); these compounds have not been identified in pheromones of its prey. In general, pheromones of insect pests can be expected to have minimal effects on nontarget insects.

EXPOSURE

Exposure of non-target organisms to a formulated pheromone will depend largely on the rate at which the material is applied, the number of applications, and the release rate of the pheromone from the formulation used. Application rates used for mating disruption vary considerably, and application rates for the active ingredient (AI) of a broadcast formulation are generally lower than those for individually placed dispensers; however, it must be taken into account that multiple applications of broadcast formulations are usually used in a season, while individually placed dispensers generally give season-long control. A review of uses of pheromones to control lepidopteran pests indicates relatively low application rates when compared to many conventional insecticides. For example, application rates given on product labels for 9 pheromone products used against 6 lepidopteran insects range from 1 g per hectare (0.4 g per acre) to 78 g per hectare (31 g per acre) per application (Ridgway & Inscoc, this volume).

ENVIRONMENTAL FATE

Persistence

Henson (1977) reported studies showing that the half-life of gossyplure, the two-component pheromone of the pink bollworm, on moistened soil was 1 day, while in water it was 7 days. The major degradation product was the mixture of alcohols obtained by hydrolysis of the two acetates, and much of the loss was attributed to volatilization. Similarly, Shaver (1983) reported half-lives in water at 24°C of 30 hours for (Z)-9-tetradecenal and 90 hours for (Z)-11-hexadecenal; in soil, the half-lives at 22°C were 29 hours and 50 hours, respectively. They concluded that these compounds are not likely to accumulate in either soil or water.

Aerial and environmental concentrations

Because of analytical limitations, early attempts at measuring concentrations of pheromones in air after pheromone treatments required unrealistically high dosages of applied pheromone and gave mixed results (Caro 1982). With improved instrumentation, more accurate data can be expected. A new, sensitive apparatus for field measurements of aerial pheromone concentrations, with an insect antenna used as detector (Koch *et al.* 1990) was described recently. With this apparatus, differences in aerial concentrations over treated and untreated areas could be observed. When foliage was present, the aerial concentration of applied pheromone rose more slowly and also decayed more slowly when the pheromone source was removed. This was attributed to adsorption of pheromone by the leaves. These phenomena resembled those suggested by Caro (1982), who postulated that the "close resemblance of the long-chain hydrocarbon structures of many pheromones to the structures of plant leaf waxes might favor the rapid sorption of airborne pheromones on surfaces of plants. . . at night when the surfaces are cool" and desorption "the next day as solar radiation increases and both temperature and atmospheric turbulence increase as a consequence." The observations that male pea moths continued to be attracted to plants near a pheromone trap site even after the trap had been removed (Wall *et al.* 1981, Wall & Perry 1983) also suggest adsorption of pheromone by foliage. More study along these lines is needed before we can fully understand the behavior of pheromones in the field. However, the concentrations of any plant-adsorbed pheromone can be expected to be very low.

Degradation

In considering the effects of chemicals on the environment, another factor that must be considered is the nature of the degradation products. In the studies mentioned above, the major degradation product of gossyplure was the mixture of alcohols obtained by hydrolysis of the two acetates (Henson 1977). Twelve products were identified in a study on the partial photodegradation of (Z)-9-tetradecenal (Fig. 1) in hexane solution in a sealed tube under air (Shaver &

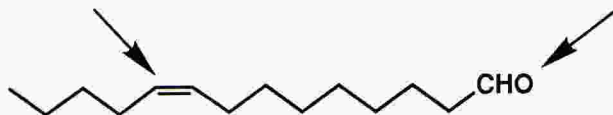


Fig. 1. Structure of (Z)-9-tetradecenal. Arrows indicate reactive sites.

Ivie 1982): *cis*- and *trans*-3-butyloxiraneoctanal [9,10-epoxytetradecanal], *cis*- and *trans*-3-butyloxiraneoctanoic acid [9,10-epoxytetradecanoic acid], *cis*- and *trans*-3-butyloxiraneheptanal [8,9-epoxytridecanal], *cis*- and *trans*-2-butyl-3-heptyl-oxirane [5,6-(= 8,9)-epoxytridecane], (Z)-9-tetradecanoic acid, (Z)-8-tridecenal, (Z)-5-tridecene, and (Z)-5-dodecene. Similar products were obtained from (Z)-11-hexadecenal under the same conditions. The nature of these products suggests that the major mechanisms of degradation under these conditions were

oxidation of the double bond to an epoxide, oxidation of the -CHO to -COOH, and subsequent decarboxylation. Further degradation of these products would be expected, giving short-chain compounds similar to ones present in most living organisms. Subsequent studies showed that the products in water or soil were generally the same as those in hexane.

Attempts have been made to measure residues of pheromone components in fruit treated with various lepidopteran pheromone formulations, but no detectable residues have been found (Spittler *et al.* 1988, this volume).

Other lepidopteran pheromones

Not all lepidopteran attractant pheromones are emitted by the females. Numerous male-emitted sex pheromones have been identified (Tamaki 1988), but the majority of these are stimulatory, rather than attractive. Tamaki (1988) notes that short-range attraction of female moths by male-emitted pheromone components has been reported for five insect species. Recent findings with the cabbage looper (*Trichoplusia ni*) show a different effect. The major component of the pheromone emitted by the female of this species was the first pheromone of a lepidopteran pest to be identified (Berger 1966); other components have since been identified. Landolt & Heath (1989, 1990) have now shown that the mating behaviour of this insect also involves long-range attraction of the female to the male and that this attraction is enhanced by host-plant kairomones. They identified the attractive components of the male pheromone as *d*-linalool, and *m*- and *p*-cresols (Fig. 2). Data in Table 5 show that these compounds are

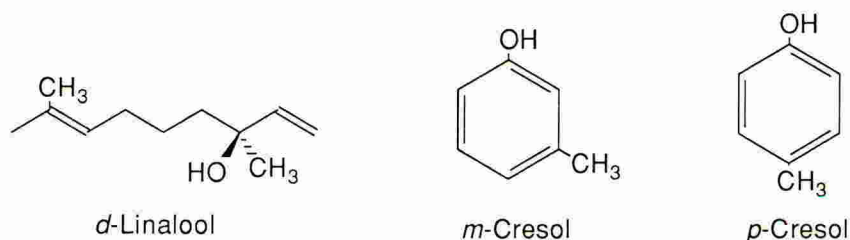


Fig. 2. Compounds produced by male cabbage loopers (From Landolt & Heath 1990).

significantly more toxic than the lepidopteran pheromone groups being considered in this paper, and the two cresols are classed as corrosive (US National Institute of Occupational Safety and Health 1982). If a commercial use were found for this pheromone, application of EPA's tier-testing scheme might well result in toxicity data requirements for registration that were considerably more stringent than those for some other pheromones. Therefore, generalizations about the relative safety of groups of pheromones should be based on carefully defined classes of chemicals for which toxicological data are available.

CONCLUSION

From the available reports, all indications are that the long-chain alcohols, acetates, and aldehydes that make up the majority of lepidopteran female sex pheromone components are essentially innocuous to non-target species and to the environment. Therefore, reduction or elimination of toxicological data requirements for these compounds through standardized waivers, particularly for experimental use permits and for temporary exemptions from tolerance, would be appropriate.

REFERENCES

- Albert, A. (1968) *Selective Toxicity*. London: Methuen & Co. Ltd. 531 pp. (pp. 235-236).
- Aldrich, J.R.; Kochansky, J.P.; Abrams C.B. (1984) Attractant for a beneficial insect and its parasitoids: Pheromone of the predatory spined soldier bug, *Podisus maculiventris* (Hemiptera: Pentatomidae). *Environmental Entomology* **13**, 1031-1036.
- Bakke, A.; Lie, R. (1989) Mass trapping. In: *Insect Pheromones in Plant Protection*. A.R. Jutsum and R.F.S. Gordon (Eds). Chichester: John Wiley & Sons, Ltd. pp. 67-87.
- Berger, R.S. (1966) Isolation, identification, and synthesis of the sex attractant of the cabbage looper, *Trichoplusia ni*. *Annals of the Entomological Society of America*. **59**, 767-771.
- Beroza, M.; Inscoc, M.N.; Schwartz, P.H., Jr.; Keplinger, M.L.; Mastri, C.W. (1975) Acute toxicity studies with insect attractants. *Toxicology & Applied Pharmacology* **31**, 421-429.
- Cannon Laboratories, Inc. (1979) Acute inhalation toxicity study of cis-11-hexadecenal. Laboratory Number 9E-4924. Unpublished report submitted to the Agricultural Research Service, USDA, May 17, 1979.
- Cannon Laboratories, Inc. (1980) Acute inhalation toxicity study of Z-9-tetradecenal. Laboratory Number OF-7353. Unpublished report submitted to the Agricultural Research Service, USDA, August 12, 1980.
- Caro, J.H. (1982) The sensing, dispersion, and measurements of pheromone vapors in air. In: *Insect Suppression with Controlled Release Pheromone Systems, Vol. 1*. A.F. Kydonieus & M. Beroza (Eds). Boca Raton, FL: CRC Press. pp. 145-158.
- Daterman, G.E. (1990) Pheromones for managing coniferous tree pests in the United States, with special reference to the western pine shoot borer. In: *Behavior-Modifying Chemicals for Insect Management: Applications of Pheromones and Other Attractants*. R.L. Ridgway, R.M. Silverstein & M.N. Inscoc (Eds). New York: Marcel Dekker. pp. 317-343.
- Daughtrey, W.C.; Eutermoser, M.; Thompson, S.W.; Biles, R.W. (1989a) A subchronic toxicity study of octyl acetate in rats. *Fundamental and Applied Toxicology* **12**, 313-320.
- Daughtrey, W.C.; Smith, J.H.; Hinz, J.P.; Biles, R.W. (1990) Subchronic toxicity evaluation of tridecyl acetate in rats. *Fundamental and Applied Toxicology* **14**, 104-112.
- Daughtrey, W.C.; Wier, P.J.; Traul, K.A.; Biles, R.W.; Egan, G.F. (1989b) Evaluation of the teratogenic potential of octyl acetate in rats. *Fundamental and Applied Toxicology* **13**, 303-309.
- Henson, R.D. (1977) Environmental fate of gossypure. *Environmental Entomology* **6**, 821-822.
- Hodosh, R.J.; Keough, E.M.; Luthra, Y. (1985) Toxicological evaluation and registration requirements for biorational pesticides. In: *Handbook of Natural Pesticides: Methods, Vol. 1. Theory, Practice, and Detection*. N.B. Mandava (Ed). Boca Raton, FL: CRC Press. pp. 231-272.
- Inscoc, M.N. (1982) Insect attractants, attractant pheromones, and related compounds. In: *Insect Suppression with Controlled Release Pheromone Systems, Vol. II*. A.F. Kydonieus & M. Beroza (Eds). CRC Press, Boca Raton, FL. pp. 201-295.
- Inscoc, M.N.; Leonhardt, B.A.; Ridgway, R.L. (1990) Commercial availability of insect pheromones and other attractants. In: *Behavior-Modifying Chemicals for Insect Management: Applications of Pheromones and Other Attractants*. R.L. Ridgway, R. M. Silverstein & M.N. Inscoc (Eds). New York: Marcel Dekker. pp. 631-715
- Kirsch, P. (1988) Pheromones: Their potential role in control of agricultural insect pests. *American Journal of Alternative Agriculture* **3**, 83-97.
- Knipling, E.F. (1976) Role of pheromones and kairomones for insect suppression systems and their possible health and environmental impacts. *Environmental Health Perspectives* **14**, 145-152.
- Koch, U.T. (1990) A portable EAG system for measurement of pheromones in the field. *Abstracts of Papers, 200th National Meeting of the American Chemical Society, Washington, DC, August 1990, Part 1*. AGFD 31.
- Landolt, P.J.; Heath, R.R. (1989) Attraction of female cabbage looper moths (Lepidoptera: Noctuidae) to male-produced sex pheromone. *Annals of the Entomological Society of America* **82**, 520-525.

- Landolt, P.J.; Heath, R.R. (1990) Sexual role reversal in mate-finding strategies of the cabbage looper moth. *Science* **249**, 1026-1028.
- The Merck Index*, 10th Edition (1983) Rahway, NJ: Merck & Co., Inc.
- Shaver, T. N. (1983) Environmental fate of (Z)-11-hexadecenal and (Z)-9-tetradecenal, components of a sex pheromone of the tobacco budworm (Lepidoptera: Noctuidae). *Environmental Entomology* **12**, 1802-1804.
- Shaver, T.N.; Ivie, G. W. (1982) Degradation products of (Z)-11-hexadecenal and (Z)-9-tetradecenal, components of a sex pheromone of the tobacco budworm. *Journal of Agricultural and Food Chemistry* **30**, 367-371.
- Spittler, T.D.; Leichtweis, J.C.; Dennehy, T.J. (1988) Biorational control of crop pests by mating disruption: Residue analyses of Z-9-dodecen-1-yl acetate and Z-11-tetradecen-1-yl acetate in grapes. In: *Biotechnology for Crop Protection*. P.A. Hedin, J.J. Menn & R.M. Hollingworth (Eds), *ACS Symposium Series* **379**, 430-436.
- Tamaki, Y. (1988) Pheromones of the Lepidoptera. In: *CRC Handbook of Natural Pesticides. Volume IV. Pheromones*. Part A. E.D. Morgan & N.B. Mandava (Eds). Boca Raton, FL: CRC Press. pp. 35-94.
- Teeters, W.R. (1979) Series of untitled reports submitted by US EPA to the Agricultural Research Service, USDA, on March 9, 1979; May 9, 1979 (4 reports); May 10, 1979; July 19, 1979. Unpublished.
- Tinsworth, E.F. (1990) Regulation of pheromones and other semiochemicals in the United States. In: *Behavior-Modifying Chemicals for Insect Management: Applications of Pheromones and Other Attractants*. R.L. Ridgway, R.M. Silverstein & M.N. Inscoe (Eds). New York: Marcel Dekker. pp. 569-603.
- US National Institute of Occupational Safety and Health (1982) *Registry of Toxic Effects of Chemical Substances*, Rockville, MD: US Department of Health and Human Services, National Institute of Occupational Safety and Health.
- Wall, C.; Perry, J.N. (1983) Further observations of the responses of male pea moth, *Cydia nigricana*, to vegetation previously exposed to sex-attractant. *Entomologia Experimentalis et Applicata* **33**, 112-116.
- Wall, C.; Sturgeon, D.M.; Greenway, A.R.; Perry, J.N. (1981) Contamination of vegetation with synthetic sex-attractant released from traps for the pea moth, *Cydia nigricana*. *Entomologia Experimentalis et Applicata* **30**, 111-115.
- Worthing, C.R. (1979) *The Pesticide Manual*, 6th Ed. Croydon: The British Crop Protection Council.

USE PATTERNS AND NON-TARGET EFFECTS OF FOREST COLEOPTERAN SEMIOCHEMICALS

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ABSTRACT

Semiochemicals are becoming increasingly valuable tools in the integrated management of forest pests. In management of coleopteran pests, they are used chiefly in traps for monitoring and timing of applications of insecticides and other control measures and as tree baits to contain and concentrate infestations so the insects can be more easily destroyed. Use of coleopteran antiaggregation pheromones as mating disruptants is also under investigation. Known forest coleopteran semiochemicals are, for the most part, naturally occurring monoterpenes and their derivatives, as well as cyclic ketals and acetals. Some of these behaviour-modifying compounds are synthesized by the insects directly, while others are of plant origin, emitted by trees or are ingested by these insects and emitted in the frass. In general, these compounds fall into one of the two lowest toxicity categories established for pesticide labelling purposes in the United States (US). For instance, rat oral LD₅₀s reported for α - and β -pinene, myrcene, camphor, and a mixture of *exo*-brevicomin, frontalin and myrcene were greater than 2,000 mg/kg, while dermal LD₅₀s on rabbits were greater than 5,000 mg/kg. A comparison of the estimated atmospheric concentrations of semiochemicals produced by an infestation of mountain pine beetles, *Dendroctonus ponderosae*, with the calculated emission rates of the chemicals from controlled-release formulations used as tree baits showed that the use of these semiochemicals as tree baits, unlike the use of sex pheromones as mating disruptants, would not be likely to increase the environmental concentration above that which could be expected to occur naturally in areas experiencing infestation levels that are high enough to justify attempts at control. Accordingly, the exemption of pheromone or semiochemical traps from US pesticide registration requirements has appropriately been applied to the use of tree baits in the management of the mountain pine beetle. Conversely, formulations of antiaggregation pheromones, like pheromone formulations used in mating disruption, are subject to registration as biochemical pesticides in the US, Canada, and many other countries. However, the available data on non-target effects indicate that minimum risk is likely to be associated with their use as antiaggregants.

INTRODUCTION

"Semiochemical" is an encompassing term applied to a broad array of naturally occurring chemicals used by organisms to perceive and communicate with other organisms. Semiochemicals have been subdivided according to their mode of action and organisms affected (Nordlund 1981). Prominent among the divisions are pheromones, allomones, and kairomones (Figure 1). Semiochemical-mediated interactions among organisms in an ecosystem can be extremely complex, as summarized for bark beetles and their host, predator, and commensal species (Figure 2).

Beetles in many coleopteran species cause significant damage to agricultural crops and forest resources (Mitchell 1981; Forestry Canada 1989, US Forest Service 1989). Damage to living conifers by bark beetles can be extensive. For example, the mountain pine beetle (MPB), *Dendroctonus ponderosae*, annually kills tens to hundreds of millions of pine trees in western Canada and western US. In the early 1980s, when infestations were at their highest levels,

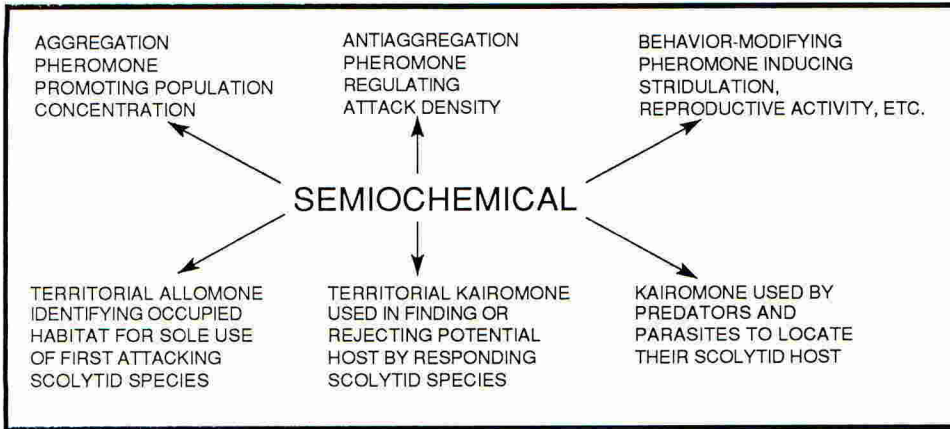


Figure 1. Examples of intraspecific and interspecific bark beetle semiochemicals (Borden 1982).

damage to the British Columbian economy was estimated to be over \$400 million annually (Manning 1982). Their deleterious impact has proved to be a major stimulus to identify the semiochemicals MPB depends on for survival and to explore ways to integrate synthetic duplicates of these chemicals into pest management strategies and tactics.

Research and development efforts involving bark beetle semiochemicals have concentrated on host-related chemicals, beetle-released pheromones, and antiaggregation pheromones (sometimes considered mating disruptants) (Borden & Lindgren 1988). Applications of various semiochemicals have included use in traps for monitoring and mass trapping, as tree baits, and as antiaggregants to prevent mass attack on trees.

Since 1983 (US Environmental Protection Agency 1983), semiochemical traps have been exempted by the US Environmental Protection Agency (US EPA) from registration as pesticides (pest control products) under the Federal Insecticide Fungicide and Rodenticide Act (FIFRA), as long as certain criteria that address the basic concern for human and environmental protection are met. These criteria were stated in the US *Code of Federal Regulations*, as follows:

"§162.5 Pesticides required to be registered.

* * * * *

"(d) *Exemption from the requirements of FIFRA.* The following pesticides or classes of pesticides are exempted from the provisions of FIFRA, when used in the manner specified: ...

...

"(2) *Pesticides of a character which are unnecessary to be subject to FIFRA.* Pheromones and identical or substantially similar compounds labelled for use only in pheromone traps and pheromone traps in which those compounds are the sole active ingredient(s).

...

"(iv) For the purposes of this paragraph a pheromone trap is a device containing a pheromone or identical or substantially similar compound that:

"(A) Is used for the sole purpose of attracting and trapping or killing target arthropods;

"(B) Achieves pest control by removal of target organisms from their natural environment; and

"(C) Does not result in increased levels of pheromones or identical or substantially similar compounds over a significant fraction of a treated area." (US Environmental Protection Agency 1983).

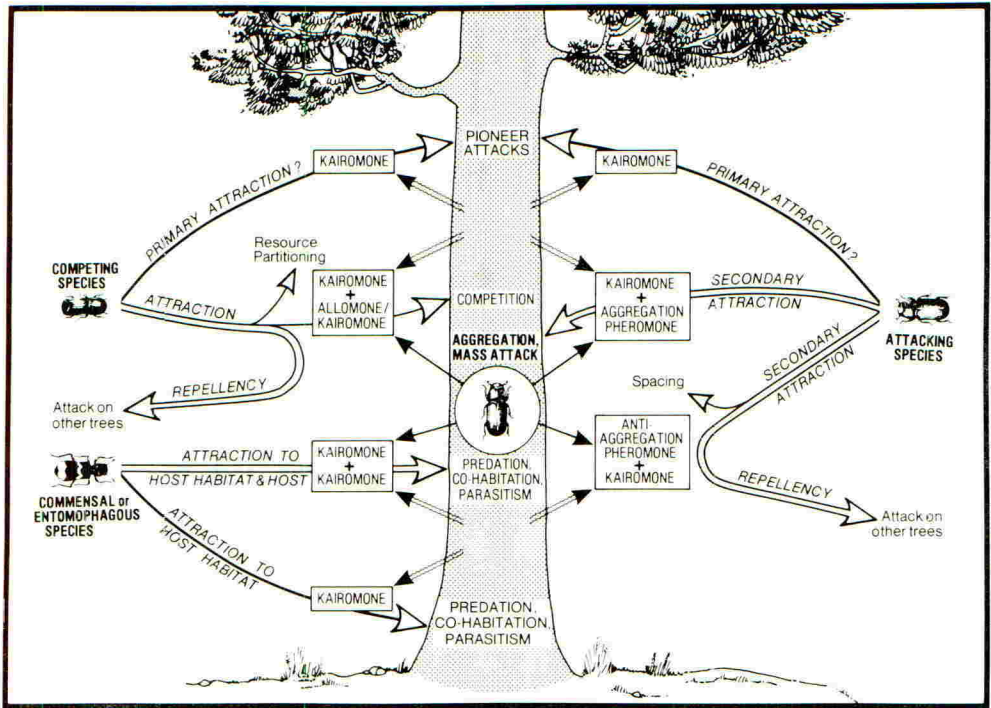


Figure 2. Semiochemical interactions of an attacking species of bark beetle with its host and other insect species (Borden 1989).

Other strategies for semiochemical use, such as mating disruption, crop baiting and repulsion, are viewed with greater concern by US EPA, in part because of factors such as high field dosages, high numbers of release devices in a treatment area, and extensive use (US Environmental Protection Agency 1983). Of key concern are the toxicological effects that increased exposure may have on humans, especially as a result of application and handling, and on other organisms in the environment where the products are intended to be used.

USE PATTERNS

Monitoring and mass trapping

Coleopteran semiochemicals are used in traps principally for monitoring purposes, although mass trapping strategies have been used against a few Coleoptera as part of integrated pest management programs (Table 1). For monitoring, relatively few traps are needed. Widespread intensive use of traps, as in the case of ambitious mass trapping programs, may well be precluded by cost factors.

Tree baits

Tree baits containing attractive host kairomones and insect pheromones are used to contain and concentrate known infestations of bark beetles (Gray & Borden 1989, Borden 1990).

TABLE 1. Applications of semiochemicals in management of coleopteran pests—selected examples (from Inscoe *et al.* 1990, Ridgway *et al.* 1990).

Sector	Pest insect example	Semiochemical use
Agriculture		
	Field	Western corn rootworm <i>Diabrotica virgifera</i>
	Cotton boll weevil <i>Anthonomus grandis</i>	Monitoring/Mass trapping
Stored grain	Khapra beetle <i>Trogoderma granarium</i>	Monitoring
	Lesser grain borer <i>Rhyzopertha dominica</i>	Monitoring
Forest		
	Field	Mountain pine beetle <i>Dendroctonus ponderosae</i>
	Douglas-fir beetle <i>Dendroctonus pseudotsugae</i>	Monitoring
Storage	Ambrosia beetles <i>Trypodendron lineatum</i>	Monitoring/Mass trapping
	<i>Gnathotrichus sulcatus</i>	

Flying beetles attracted to the baited tree colonize the tree and release additional pheromones. This emission eventually overrides the effect of the bait, attracting more beetles and causing mass attack on the baited trees and surrounding trees. Attracted beetles can then be controlled by destroying the attacked trees in place, *e.g.*, by felling and burning, or by logging the attacked trees.

Tree baits containing monoterpenoid and bicyclic ketal semiochemicals have been developed for four *Dendroctonus* bark beetle species (Table 2). These baits are used primarily to contain and concentrate known infestations until selective or clear-cut logging removes the infested trees. Log processing kills resident beetles by destroying the cambial tissues where they feed and breed. The size of the harvested area is effectively reduced when this strategy is employed because tree baits cause infestations to intensify in a limited area, rather than to expand. Moreover, tree baits are characteristically utilized in industrial forestry situations where bark-beetle infested areas are already a high priority for selective and clear-cut harvesting (Borden 1990).

Deployment strategies for tree baits vary, depending on the size and location of infestations (Phero Tech 1986a, 1986b, 1986c). Small infestations, where fewer than 30 trees contain maturing beetles, are "spot baited." Baits are applied to two or three trees in the centre of each spot. For larger infestations or a localized concentration of small spots, trees are baited at 50-meter intervals on a grid, at least 25 meters within the boundaries of the infested area. In the largest infestations, baits are arranged in two or three barrier lines, with trees baited at 50-meter intervals within the perimeter of the infested area. The lines are staggered so that flying beetles attempting to cross the barrier must fly within 25 meters of a baited tree. The outermost line is set at least 25 meters within the infestation boundaries. Baits are placed prior to anticipated summer attack and last approximately 90 days. Attacked trees are harvested prior to the next flight (the following summer). In the year following removal of the attacked trees, a few fresh baits are affixed to standing mature pines within and immediately surrounding the management site to remove or "mop up" any remaining beetles.

TABLE 2. Semiochemicals in tree-bait products for four forest coleopteran pests.

Pest species	Active ingredients in tree baits
Douglas-fir beetle <i>Dendroctonus pseudotsugae</i>	frontalin, α -pinene, camphene ^a
Mountain pine beetle <i>Dendroctonus ponderosae</i>	<i>trans</i> -verbenol, <i>exo</i> -brevicommin, myrcene ^b
Spruce beetle <i>Dendroctonus rufipennis</i>	frontalin, α -pinene ^c
Western balsam bark beetle <i>Dryocoetes confusus</i>	<i>exo</i> -brevicommin ^d

^a Phero Tech 1986a. ^b Borden 1990. ^c Phero Tech 1986b.

^d Phero Tech 1986c.

Antiaggregation pheromones

When the population of bark beetles in a tree under mass attack reaches a sufficiently high level, many bark beetles release antiaggregation pheromones that interrupt the response of other attacking beetles to the attractive semiochemicals. These serve to prevent beetle densities from increasing to such a high level that successful reproduction could be endangered. Synthetic versions of these semiochemicals have potential as a means of preventing colonization of high-value individual trees, protecting uninfested stands from attack, and reducing infestation levels in stands that have already incurred some attack. One of the earliest of discovered antiaggregation pheromones was methylcyclohexenone (MCH), a potent antiaggregant for Douglas-fir beetle, *D. pseudotsugae*, and spruce beetle, *D. rufipennis* (McGregor *et al.* 1984, Lindgren *et al.* 1989a). Another is verbenone, reported to reduce mass attacks of MPB (Ryker & Yandell 1983, Lindgren *et al.* 1989b), western pine beetle, *D. brevicomis* (Bedard *et al.* 1980), and southern pine beetle, *D. frontalis* (Rudinsky 1973). Antiaggregation pheromones continue to be investigated for their ability to disrupt mass attacks, thereby reducing beetle survival and reproduction.

Antiaggregants are released from many small point sources (roughly 100,000/hectare) or from "bubble cap" release devices (from 100 - 150/hectare). Typical dosages range from 80 to 150 g/hectare. Both MCH and verbenone have been field tested, with promising results (Furniss *et al.* 1977; McGregor *et al.* 1984; Amman *et al.* 1989; Lindgren *et al.* 1989a,b); further investigation continues.

CHEMISTRY AND NATURAL OCCURRENCE OF MONOTERPENES AND CYCLIC KETALS

Monoterpenes, the active compounds in many bark beetle semiochemicals, are monoterpenes or cyclic acetals and related compounds such as bicyclic ketals; all contain only carbon, oxygen and hydrogen. The chemical structures of some of those most commonly used are shown in Figure 3. For most semiochemicals, purity is critical for efficacy. As a result, synthetic semiochemicals used in practice are of such a purity as to be essentially identical to their natural counterparts.

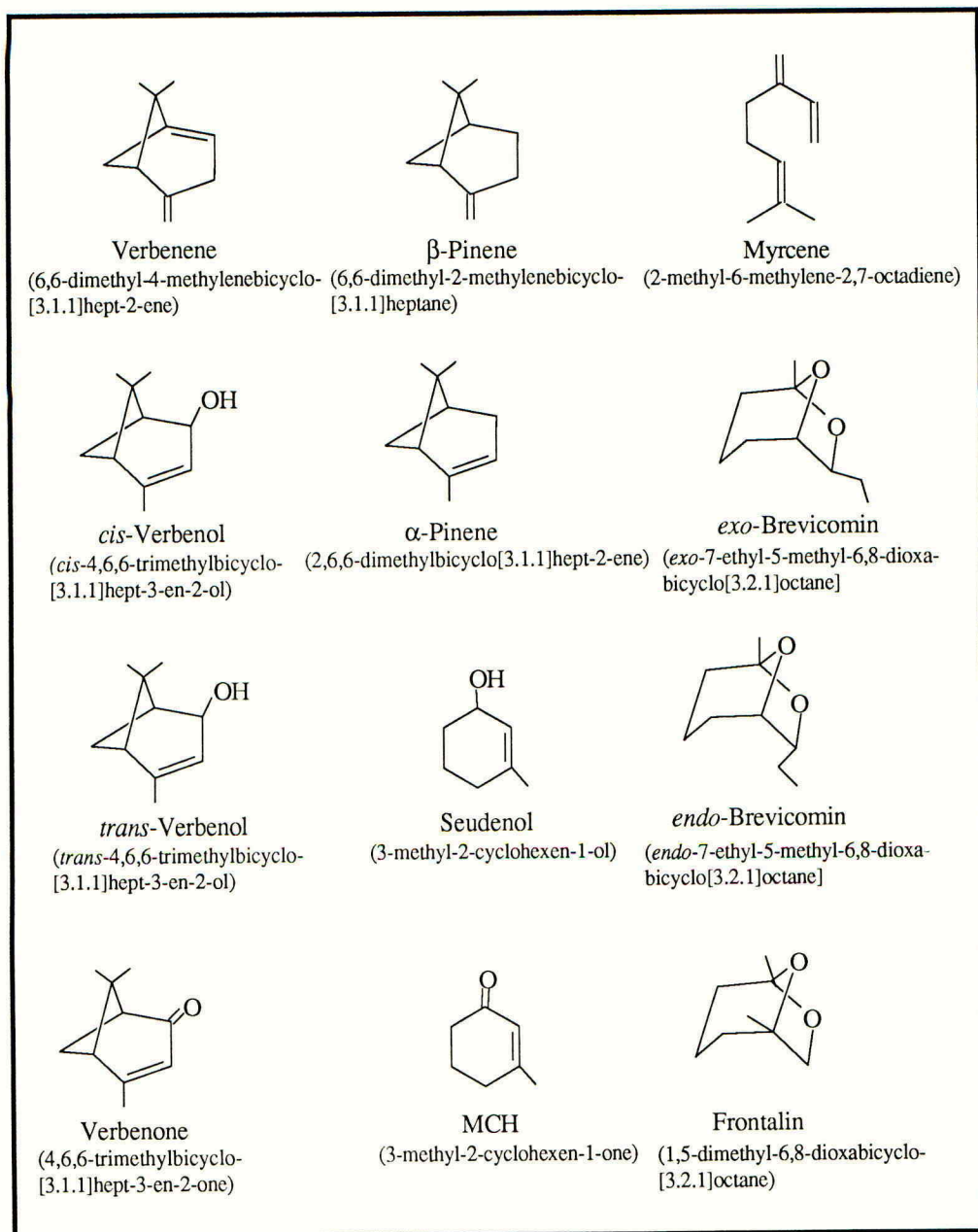


Figure 3. Examples of monoterpenes and related compounds having activity as semiochemicals for forest Coleoptera.

Many forest semiochemicals, particularly the ones released at highest rates (*e.g.*, myrcene), are relatively simple monoterpenes; these are made biosynthetically from two isoprene units. Many monoterpenes see everyday use in flavouring agents, perfumes, deodorizers, solvents, and other similar products (Bedoukian, this volume) and as a consequence, their toxic effects on mammals are reasonably well understood. This wide-spread use suggests that these chemicals, as a class, are relatively non-toxic.

Cyclic ketals pheromone components such as *exo*-brevicomin are normally released at very low rates in comparison to host volatile monoterpenes. Although they are relatively simple molecules, they are structurally somewhat more complex than monoterpenes. They are usually synthesized by the insects themselves (Vanderwel & Oehlschlager 1989) and thus are less prevalent than the terpenes in the natural environment. Few, if any, see common use by man, so background chronic exposure is limited. The following discussion focuses on the more familiar and more utilized naturally occurring monoterpenes.

Monoterpenes, together with the closely related sesquiterpenes (composed of three or more isoprene units), and their oxidized derivatives, constitute the documented major resin components in over 50 plant families (Croteau 1986, Hillis 1987). Isoprene and monoterpenes are prevalent in the forest atmosphere, although concentrations can vary with time of day and year (Yokouchi *et al.* 1983, Isidorov *et al.* 1985). Analysis of monoterpenes collected from air within a Scandinavian forest composed primarily of Scots pine showed high levels of α -pinene, β -pinene, and limonene and lower levels of many other familiar terpene chemicals (Petersson 1988) (Table 3). Total monoterpene concentrations were in the order of 100 mg/m³, or roughly 10 g/hectare, assuming a height of 10 meters. Additional studies in Russia identified similar monoterpenes (Isidorov *et al.* 1985), although the calculated atmospheric concentrations were significantly lower than those found by Petersson (1988) in Scandinavia.

TABLE 3. Concentrations of major monoterpenes in summer night air in a Scandinavian plantation of Scots pine (Petersson 1988).

Compound ^a	Concentration in $\mu\text{g}/\text{m}^3$	
	June 20, 1983	July 8, 1983
α -Pinene	54	23
Camphene	3.9	2.7
β -Pinene	1.8	1.4
Myrcene	1.2	1.4
3-Carene	39	27
α -Phellandrene	2.2	1.5
Limonene	7.3	1.9

^a Concentrations of α -thujene, sabinene, α -phellandrene, γ -terpinene, terpinolene and *p*-cymene were below 1 $\mu\text{g}/\text{m}^3$.

Loblolly pine, found across much of the southern US, produces similar monoterpenes. When subjected to inoculations of the blue stain fungus, *Ceratocystis minor*, which often accompanies southern pine beetle, monoterpene production in loblolly pine increased forty-fold in an apparent defensive response, but no new monoterpenes were detected (Gambliel *et al.* 1985). When lodgepole pine resin was analyzed, monoterpenes were prominent components, *e.g.*, β -phellandrene (69%), myrcene (3.9%), and α -pinene (6.4%) (Smith 1964). Essential oils from table mountain pine also contained common monoterpenes such as α - and β -pinenes and some less common ones such as γ -muurolene and α -bisabolol (Table 4).

TABLE 4. Relative composition (%) of table mountain pine (*Pinus pungens*) needle oil components (Ekundayo 1980).

Compound	Relative %
α -Pinene	4.8
Camphene	0.9
β -Pinene	18.3
Myrcene	1.0
3-Carene	1.4
Unidentified C ₁₀ hydrocarbon	1.2
<i>p</i> -Cymene	1.3
Limonene	2.3
β -Phellandrene	1.0
Terpinolene	0.8
Unidentified C ₁₀ alcohol	0.5
α -Terpineol	0.6
Bornyl acetate	2.0
Geranyl acetate	0.1
Unidentified C ₁₅ hydrocarbon	2.2
Caryophyllene	5.4
Humulene	3.2
γ -Muurolene	16.0
β -Bisabolol	8.1
δ -Cadinene	9.2
α -Bisabolol	14.7
α -Cadinol	1.8
δ -Cadinol	2.4
Unidentified C ₁₅ alcohol	0.8

Although many monoterpenes are prevalent in the natural forest environment, the proportionately higher concentration of a select few terpenes such as the pinenes and their oxidized derivatives suggests some common and simple biosynthetic and degradation pathways. This commonality is supported by an extensive body of literature, accumulated particularly in the last 40 years. Many semiochemically active compounds are key degradation products of prominent monoterpenes. α -Pinene, itself a kairomone, spontaneously oxidizes to form *trans*-verbenol, *trans*-pinocarveol, verbenone, and a number of other closely related derivatives (Smith 1964, Moore *et al.* 1955), and the natural interconversion of α -pinene, *trans*-verbenol, and verbenone has been demonstrated (Hunt *et al.* 1989). Studies on bacterial degradation of α -pinene showed that metabolites were formed through relatively simple oxidation and ring cleavage reactions (Griffiths *et al.* 1987, Gibbon & Pirt 1971).

NON-TARGET EFFECTS OF MONOTERPENES

There are some reports of undesirable effects caused by monoterpenes, for example, camphor poisoning of children through the ingestion of common cough medicines (Gibson *et al.* 1989), and dermal irritation by geraniol (3,7-dimethyl-2,6-octadien-1-ol) and citral (3,7-dimethyl-2,6-octadienal) through contact with the peelings of citrus fruits (Cardullo *et al.* 1989). The potential for toxic effects due to inhalation of turpentine, a mixture of monoterpenes, has been known for some time (Anonymous 1967). Some immunosuppressive effects of *d*-limonene have

also been documented (Evans *et al.* 1987). Despite these occasional manifestations of toxicity, monoterpenes in general have been found to have relatively low toxicological properties, particularly in consideration of their prevalent use. The reported toxicities of common semiochemical monoterpenes (Table 5) place these chemicals in the least toxic categories, III and IV, of the categories used by US EPA for labelling purposes (US *Code of Federal Regulations* 1990a) (Table 6).

TABLE 5. Toxicities of some coleopteran semiochemicals.

Compound	Oral LD ₅₀ (g per kg)	Dermal LD ₅₀ (g per kg) (rabbit)	Inhalation LD ₅₀ (mg/L) (minimum)	Eye irritation (Draize score)	Skin irritation (Draize score)
α -Pinene ^a	3.7 (rat)	>5	364-572	—	moderate
Myrcene ^{b,c}	>5 (rat)	>5	—	—	low-moderate
β -Pinene ^a	4.7 (rat)	>5	19.6	—	—
<i>exo</i> -Brevicommin, frontalin, and myrcene ^d	2.24 (rat) 2.7 (mouse)	5.1	>100	—	0
(+)-Camphora ^a	>5 (rat) 1.3 (mouse)	>5	—	—	moderate
3-Methyl-2- cyclohexenone (MCH) ³	1-2 (rat)	—	>19.7	0	—
Verbenone ^f	1.8-3.4 (rat)	—	2.0	0	0

^a Opdyke 1978, p. 853. ^b Opdyke 1976, p. 615. ^c US National Institute of Occupational Safety and Health 1988. ^d US Forest Service 1991a. ^e US Forest Service 1991b. ^f Phero Tech 1987.

Some quantitative information pertaining to effects of semiochemical monoterpenes and related chemicals on non-target organisms other than mammals is available. Pinane (saturated α - or β -pinene) has been reported to be mildly toxic to *Daphnia* but could cause sublethal effects at concentrations as low as 0.1 ppm (Savino & Tanabe 1989). Pristane (2,6,10,14-tetramethylpentadecane), a higher terpene, was found to accumulate quickly in fish lipids but was rapidly metabolized (Le Bon *et al.* 1987). Some effects of pristane on trout growth

TABLE 6. Toxicity categories designated by the US Environmental Protection Agency for labelling purposes (US Code of Federal Regulations 1990a).

Hazard indicators	Toxicity Categories			
	I	II	III	IV
Oral LD ₅₀	Up to and including 50 mg/kg	From 50 through 500 mg/kg	From 500 through 5,000 mg/kg	Greater than 5,000 mg/kg
Inhalation LC ₅₀	Up to and including 0.2 mg/liter	From 0.2 through 2 mg/liter	From 2 through 20 mg/liter	Greater than 20 mg/liter
Dermal LD ₅₀	Up to and including 200 mg/kg	From 200 through 2,000 mg/kg	From 2,000 through 20,000 mg/kg	Greater than 20,000 mg/kg
Eye effects	Corrosive; corneal opacity not reversible within 7 days	Corneal opacity reversible within 7 days; irritation persisting for 7 days	No corneal opacity; irritation reversible within 7 days	No irritation
Skin effects	Corrosive	Severe irritation at 72 hours	Moderate irritation at 72 hours	Mild or slight irritation at 72 hours

following chronic exposure have been reported (Luquet *et al.* 1984). In a study on 3-methyl-2-cyclohexenone (MCH), the subacute dietary LC₅₀ in quail chicks was >40,000 ppm, and the 96-hr LC₅₀ values were 1 ppm and 72 ppm in bluegill and rainbow trout, respectively (US Forest Service 1991b). In a more recent study, the LC₅₀ values for bluegill and rainbow trout were found to be 25.3 ppm and 44.9 ppm, respectively (US Forest Service 1991c). These results show that MCH has relatively low toxicity.

A coleopteran semiochemical product, grandlure, a complex mixture of four monoterpenes, which is the synthetic version of the male-produced pheromone of the cotton boll weevil, *Anthonomus grandis* has been widely used for a number of years (Ridgway & Inscoc, this volume). Grandlure, formulated in controlled-release dispensers, is used in traps to improve targeting and timing of controls and for mass trapping. Grandlure was first used prior to the regulatory exemptions of pheromones labelled for use as the sole active ingredient in traps, and therefore toxicological data for the mixture of the four grandlure components were obtained for use in the regulatory process (Hedin *et al.* 1976). The results were as follows: oral LD₅₀ in mice, >600 mg/kg; dermal LD₅₀ in rabbits, >500 mg/kg; intravenous LD₅₀ in mice, 100 mg/kg; mild skin irritant to rabbits; moderately irritating to eye of rabbits; 96-hr LC₅₀ for bluegill sunfish, 44 ppm; 8-day dietary LC₅₀ for mallard ducklings and bobwhite quail chicks, >5000 ppm. These data failed to reveal any non-target effects of potential concern.

REGULATORY ISSUES

Tree baits

Tree baits are used to manage and contain bark beetle populations and may therefore be defined under US law as pesticides, subject to regulation under FIFRA. Similarly, because they are used to "mitigate" pest populations, they are regarded in Canada as pest control agents and are subject to regulation under the *Pest Control Products Act (Revised Statutes of Canada 1985)*. Major requirements, when registration is required in the US, include data on mammalian toxicity and non-target effects for each biologically active chemical compound involved. Thus, for registration of the MPB tree bait, data would be needed for the three active ingredients, myrcene, *exo*-brevicomin, and *trans*-verbenol. Considerable data for myrcene have already been published (Table 5). Some toxicological information also exists for a mixture containing *exo*-brevicomin (Table 5); however, both myrcene and frontalin (a close relative of *exo*-brevicomin) were present with *exo*-brevicomin in the tested material, so information on *exo*-brevicomin alone is not available. Although little published toxicological information is available on *trans*-verbenol, data are available on a closely related compound, the well known monoterpene, α -pinene, which has an oral LD₅₀ in the rat of 3.7 g/kg (Table 5). Although the accessible information regarding the toxicology and degradation of the active ingredients of the MPB tree baits suggests no significant problems, data are not available to meet the specific requirements for registration under FIFRA (US *Code of Federal Regulations* 1990b) without substantial use of waiver provisions.

The exemption of pheromones in traps from regulation under FIFRA (US Environmental Protection Agency 1983) and the definition of "pheromone trap" given therein (see above) seemed to offer the possibility for exemption of tree baits from regulation. Tree baits as currently designed are devices containing a pheromone as the sole active ingredient; they are "used for the sole purpose of attracting and trapping or killing target arthropods," and they "achieve pest control through removal of target organisms from their natural environment." Although the argument could be made that tree baits on their own are not the device that removes the insects, they could be considered to be the causative agent of the beetle removal, since they induce mass attack on trees, and the beetles are removed with the tree and destroyed when the trees are debarked.

The applicability to tree baits of the third part of the US EPA trap definition—"does not result in increased levels of pheromones or identical or substantially similar compounds over a significant fraction of a treated area"—is a complex issue that deserves detailed examination. Obviously, tree baits can cause the levels of semiochemicals to be increased in the areas where they are placed. However, natural background levels vary greatly over a season, and there can be large differences between semiochemical levels in low and severe infestations. Indeed, pheromone traps, which are exempted from regulation under FIFRA, can also cause significant increases in semiochemical levels over background, particularly in quarantine or monitoring situations where traps are deployed as early warning systems before the target pests are geographically or seasonally in the area. Two central questions seem apparent in considering exemption of tree baits from regulation: what should be considered normal background levels of semiochemicals, and do tree baits result in concentration levels that exceed normal levels?

Only a limited number of measurements have been made relating to the natural production of coleopteran semiochemicals in an infested area. In the absence of analyses of air in beetle-infested forests, the concentrations of the semiochemicals can only be estimated. Although such estimates contain considerable uncertainty, they can nevertheless provide valuable insight into the amounts of various chemicals that are released into the atmosphere under natural conditions. For instance, in a study on the western pine beetle, *Dendroctonus frontalis*, Browne *et al.* (1979) measured the amounts of frontalin, *exo*-brevicomin and myrcene released from infested cut bolts in the laboratory or from the wrapped section of a colonized tree. They estimated that a male beetle produced frontalin at a rate of 8.6×10^{-7} g/day, a female produced *exo*-brevicomin at

4.1 x 10⁻⁶ g/day, and 4.1 x 10⁻⁴ g/day of myrcene was released by the boring activity of a female. They estimated that a single generation of 610,000 western pine beetles in a 65-km² forest (a relatively low-density population) released 0.78, 3.7, and 370.5 g of frontalin, *exo*-brevicommin, and myrcene, respectively in a 30-day period (*i.e.*, 1.9 mg/hectare/day of the combined chemicals).

For the mountain pine beetle, Oehlschlager (1986) made estimates of the daily emission rates of the three MPB semiochemical components, *exo*-brevicommin, *trans*-verbenol, and myrcene, in a hypothetical MPB-infested lodgepole pine forest. These estimated rates, calculated as follows, are presented in column 3 of Table 7. The stand density was taken to be 750 trees/hectare, which is not uncommon. On average, a MPB infestation, over the three-week attack period, will cause a one-third annual mortality rate (Cole & Amman 1980), so 250 trees/hectare would be killed in this hypothetical forest. It has been reported that an attack density on the order of 40 boring holes/m² (with two beetles per hole) is required to kill a lodgepole pine tree (Raffa & Berryman 1983), while 85 boring holes/m² are common (Safranyik *et al.* 1974). Taking the average tree diameter as 0.3 m and the average attack height as 10 m, the average attack area per tree would be 9.4 m². With 85 boring holes/m², Oehlschlager (1986) calculated that 200,000 bore holes/ha (or 200,000 beetles of each sex per hectare) would cause the assumed mortality of 250 trees/hectare and went on to estimate the amounts of the three semiochemicals under consideration that would be released as a result of this density of attacking beetles, as follows:

TABLE 7. Comparison of daily emission rates of semiochemicals applied in traps or tree baits for the mountain pine beetle, *Dendroctonus ponderosae*, with estimated rates in a natural high-density infestation.

Insect	Estimated emission rates (mg/ha/day)		
	Applied material (A)	Natural infestation (N)	Ratio (A/N)
Tree baits			
<i>exo</i> -Brevicommin	0.8 ^a [0.3]	0.95 ^b [0.38]	0.8
<i>trans</i> -Verbenol	4 ^a [1.6]	35 ^b [14]	0.1
Myrcene	70.4 ^a [28.2]	190,000 ^b [76,000]	0.0004
Traps for monitoring			
<i>exo</i> -Brevicommin	0.02 ^c [0.008]	0.95 ^b [0.38]	0.02
<i>trans</i> -Verbenol	0.1 ^c [0.04]	35 ^b [14]	0.003
Myrcene	1.76 ^c [0.7]	190,000 ^b [76,000]	0.000009

^a Oehlschlager 1985, calculated for 4 baits/hectare. ^b Oehlschlager 1986.

^c From Borden 1990,1991, calculated for a trap density of 1 trap/10 hectare.

The first compound, *exo*-brevicomin, is produced almost exclusively by the male MPB and a male beetle releases about 1×10^{-4} mg of *exo*-brevicomin upon mating (Conn 1981). Thus the 200,000 male beetles/ha in the hypothetical infestation would release 20 mg/ha in total over the three-week attack period, or 0.95 mg/ha/day.

The second semiochemical, *trans*-verbenol, is produced by the female MPB. A female beetle has been conservatively estimated to produce 1×10^{-4} mg/day of *trans*-verbenol (Borden 1985); for the hypothetical infestation considered by Oehlschlager (1986), the 200,000 female MPBs would emit roughly 20 mg/ha/day. However, *trans*-verbenol arising from the air oxidation of α -pinene that is released by the attacked trees must also be considered (Borden *et al.* 1986). α -Pinene is naturally released from pine tree bark and foliage, but when the trees are heavily attacked by MPBs, it is released in greater quantities from the boring holes that the female beetles create. The injury caused by the female MPB stimulates the tree to produce large amounts of oleoresin, containing the following volatiles: β -phellandrene, 69%; myrcene, 3.9%; and α -pinene, 6.4% (Smith 1964). Using estimates of the total area of the boring holes and measurements of the volatility of α -pinene, Oehlschlager (1986) calculated that the amount of α -pinene evaporating as a result of the MPB attack would be approximately 317 g/ha/day. Air oxidation of α -pinene at room temperature has been found to produce *trans*-verbenol at an approximate rate of 46 mg/g of α -pinene/day (Borden *et al.* 1986). Accordingly, about 15 mg/ha/day of *trans*-verbenol would be produced from the α -pinene from the attacked trees. This, together with the 20 mg/ha/day emitted by the females, gives a total of 35 mg/ha/day of *trans*-verbenol resulting from the infestation.

The third MPB semiochemical compound, myrcene, makes up 3.9% of the oleoresin produced by a tree in response to injury caused by the female beetles. Using an approach similar to that used to calculate the air concentration of α -pinene, Oehlschlager (1986) calculated that approximately 190 g/ha/day of myrcene is released in the hypothetical infested lodgepole pine stand.

To bolster the argument for an exemption from regulation for tree baits, these estimates of the levels of semiochemicals in naturally infested were compared with levels to be expected in baited areas. MPB tree baits are used at a concentration of roughly 2 - 3 baits/acre (5 - 8 baits/ha); certain deployment tactics for spot infestations may require more. They are frequently used on a 50 x 50 m grid (4 baits per hectare). The release rates of the individual components of the MPB tree baits, *exo*-brevicomin, *trans*-verbenol, and myrcene, were measured as 0.2, 1.0, and 17.6 mg/bait/day, respectively, (Oehlschlager 1985). Accordingly, the approximate release rates with a placement of four baits/hectare are 0.8, 4.0, and 70.4 mg/hectare/day, respectively (Table 7, column 2).

For comparison purposes, Table 7 also includes data on the same compounds when used in monitoring traps. For this use, the release rate of the *exo*-brevicomin is reduced at least tenfold (Borden 1990). Monitoring traps are deployed at highly variable rates, depending on the purpose of monitoring and the area to be monitored, and can range from 1 trap in 10 hectares to 1 per 10 km² (Borden 1991).

For both the MPB tree baits and the monitoring traps, Table 7 shows that the estimated semiochemical concentrations resulting from the applied materials are generally much lower than those to be expected in an "average" natural infestation of the MPB. Even if there are large variances in the estimates, the ratio of the emission rates for the applied and natural materials (A/N ratio) of tree baits, like the A/N ratio of traps used for monitoring the timing of MPB attacks, are low. These calculations indicate that a broad range of tolerance with respect to field concentration, (*e.g.*, concentrations arising from 1 trap/10 ha to 10 baits/ha), could be justified, because even the highest concentrations applied would not be expected to increase semiochemical levels significantly above concentrations occurring naturally. It therefore would be inconsistent to exempt traps from registration under FIFRA while requiring registration of tree baits. This

perspective formed a large part of the reasoning behind a decision made by US EPA in 1987 (US Environmental Protection Agency 1987) to exempt the MPB tree baits from registration requirements.

Antiaggregation pheromones

Comparisons are made in Table 8 between estimated emission rates of semiochemicals applied for managing populations of various forest insect pests by mating disruption or by antiaggregation and of those of the semiochemicals occurring naturally in infestations of these pests. These data indicate that for three lepidopteran species, mating disruptants cause a 600- to 9,000-fold increase in semiochemical concentrations over the levels occurring in a high-density infestation. Field concentrations for bark beetle antiaggregation pheromones such as verbenone and MCH may well result in A/N ratios in the 0.5–5.0 range, significantly above natural levels but substantially less than for lepidopteran mating disruptants.

TABLE 8. Comparison of daily emission rates of semiochemicals applied for mating disruption or antiaggregation of some forest insect pests with estimated daily rates in natural high-density infestations.

Insect	Estimated emission rates (mg/ha/day)		
	Applied material (A)	Natural infestation (N)	Ratio (A/N)
Western spruce budworm ^a <i>Choristoneura occidentalis</i>	175 [70]	0.18 [0.075]	>9,000
Gypsy moth ^b <i>Lymantria dispar</i>	150 [60]	0.23 [0.09]	>600
Western pine shoot borer ^{a,c} <i>Eucosma sonomana</i>	37.5 [15]	0.045 [0.018]	>800
Mountain pine beetle ^d <i>Dendroctonus ponderosae</i> (verbenone)	750-1000 [300-400]	300 [120]	>1.5

^a Sower 1987. ^b Schwalbe 1987. ^c Sower *et al.* 1982. ^d Phero Tech 1988.

Recognizing that current policy in the US requires that disruptants and antiaggregation pheromones be regulated under FIFRA and that there is interest in private companies and in the US Forest Service in pursuing the use of these pheromones, efforts to explore approaches to facilitating the regulatory process for these materials should continue. Semiochemicals currently of specific interest in this regard are verbenone, for management of the MPB, southern pine beetle, and western pine beetle, and MCH for Douglas-fir beetle and spruce beetle (US Forest Service 1991d). The data needs for either experimental use permits (EUPs) or full registration of these compounds are less than for semiochemicals used on food or feed crops, because use in forestry is a non-food use that does not require establishment of tolerances or exemptions from tolerances for residues in food or feed. Nevertheless, US EPA requirements for data on

toxicology and non-target effects continue to be burdensome, particularly for EUPs or for registrations which involve small acreages of relatively remote forested lands.

CONCLUSIONS

The process of registration of coleopteran tree bait, trap, and antiaggregation products with the US EPA has served to clarify thinking on a number of issues. Semiochemical-baited traps have been held exempt from registration because, among other things, their main use is to observe or monitor a pest population. Direct mitigation is not an objective in such monitoring programs. Mass trapping has been considered an offshoot of monitoring and not of concern unless synthetic semiochemical concentrations significantly exceed those in the natural environment, even through insect elimination and mitigation is occurring. Mating disruptants differ from traps, in that they will, in all likelihood, be used at concentrations significantly above natural levels. Antiaggregation pheromones are similar to mating disruptants in requiring use of concentrations above natural levels, but their mode of action is different and it appears that they can be effective at concentrations somewhat lower than those required with mating disruptants.

Tree baits lie somewhere in between traps or mating disruptants. Baits can mitigate pest populations, but they are deployed in the field like traps. In order to deal with regulatory issues for these products, assumed and somewhat arbitrary divisions that separated traps from other semiochemical products had to be reexamined. If all assumptions are put aside, one is left with US EPA's basic concern for human and environmental protection. When tree baits were examined from this basic perception, it was possible to develop clear thinking and progress in adjudication.

Based on what is known of their toxicology and use patterns, the semiochemicals of coleopteran forest pests do not seem to present a significant hazard. Consideration of the available data in the context of some of the issues involved (Sharratt 1979, Neely 1985, Parmeggiani 1987, Cothorn 1989) leads to the conclusion that semiochemical monoterpenes and cyclic ketals, if handled appropriately, should not present a problem in terms of human exposure. Similarly their environmental impact, at the doses anticipated, could be considered insignificant, if even detectable. Data on plant-produced chemicals structurally related to semiochemicals (Bedoukian, this volume) should also be of value in exploring reductions in data requirements for other coleopteran pheromones.

A general survey of pheromone regulatory actions (Jellinek & Gray, this volume) indicates that the use of scientific documentation and logic to justify requests for waivers has made it possible for a number of pheromones to be registered with considerably less data than would seem to be required on initial reading of the regulations (US *Code of Federal Regulations* 1990b). Opportunities exist to explore with US EPA and other regulatory agencies the possibilities of more extensive use of such reductions in data requirements to expedite development and use of semiochemicals.

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REFERENCES

- Amman, G.D.; Thier, R.W.; McGregor, M.D.; Schmitz, R.F. (1989) Efficacy of verbenone in reducing lodgepole pine infestation by mountain pine beetles in Idaho. *Canadian Journal of Forest Research* **19**, 60-64.
- Anonymous (1967) Hygienic guide series. Turpentine. *American Industrial Hygiene Association Journal* **28**, 297-298.
- Bedard, W.D.; Tilden, P.E.; Lindahl, K.Q., Jr.; Wood, D.L.; Rauch, P.A. (1980) Effects of verbenone and *trans*-verbenol on the response of *Dendroctonus brevicomis* to natural and synthetic attractant in the field. *Journal of Chemical Ecology* **6**, 997-1013.
- Borden, J.H. (1982) Aggregation pheromones. In *Bark Beetles in North American Conifers: A System for the Study of Evolutionary Biology*. J.B. Mitton & K.E. Sturgeon (Eds). Austin, TX: University of Texas Press. pp. 74-139.
- Borden, J.H. (1985) Personal communication, cited in Oehlschlager 1986.
- Borden, J.H. (1989) Semiochemicals and bark beetle populations: Exploitation of natural phenomena by pest management strategists. *Holarctic Ecology* **12**, 501-510.
- Borden, J.H. (1990) Use of semiochemicals to manage coniferous tree pests in western Canada. In: *Behavior-Modifying Chemicals for Insect Management: Applications of Pheromones and Other Attractants*. R.L. Ridgway, R.M. Silverstein, & M.N. Inscoe (Eds). New York: Marcel Dekker, Inc. pp. 281-315.
- Borden, J.H. (1991) Personal communication.
- Borden, J.H.; Hunt, D.W.A.; Miller, D.R.; Slessor, K.N. (1986) Orientation in forest Coleoptera: An uncertain outcome of responses by individual beetles to variable stimuli. In: *Mechanisms in Insect Olfaction*. T.L. Payne, M.C. Birch, & C.E.J. Kennedy (Eds). Oxford [Oxfordshire]: Clarendon Press. pp. 97-109.
- Borden, J.H.; Lindgren, B.S. (1988) The role of semiochemicals in IPM of the mountain pine beetle. In: *Integrated Control of Scolytid Bark Beetles*. T.L. Payne & H. Saarenmaa (Eds). Blacksburg, VA: Virginia Polytechnic Institute. pp. 247-255.
- Browne, L.E.; Wood, D.L.; Bedard, W.D.; Silverstein, R.M.; West, J.R. (1979) Quantitative estimates of the western pine beetle attractive pheromone components, *exo*-brevicomin, frontalin, and myrcene in nature. *Journal of Chemical Ecology* **5**, 397-414.
- Cardullo, A.C.; Ruskowski, A.M.; Deleo, V.A. (1989) Allergic contact dermatitis resulting from sensitivity to citrus peel, geraniol, and citral. *Journal of the American Academy of Dermatology* **21**, 395-397.
- Cole, M.E.; Amman, G.D. (1980) *Mountain Pine Beetle Dynamics in Lodgepole Pine Forests. Part I. Course of Infestation*. USDA Forest Service General Technical Report INT-89.
- Conn, J.E. (1981) *Pheromone Production and Control Mechanism in Dendroctonus ponderosae*. M.Sc Thesis, Simon Fraser University, Burnaby, B.C.
- Cothern, C.R. (1989) Some scientific judgments in the assessment of the risk of environmental contaminants. *Toxicology and Industrial Health* **5**, 479-491.
- Croteau, R. (1986) Biochemistry of monoterpenes and sesquiterpenes of the essential oils. In: *Recent Advances in Botany, Horticulture, and Pharmacology*. L.E. Craker & J.E. Simon (Eds). Volume 1. *Herbs, Spices and Medicinal Plants*. Phoenix, AZ: Oryx Press. pp. 81-133.
- Ekundayo, O. (1980). Essential oils III: Analysis of table mountain pine (*Pinus pungens* Lamb) needle oil by gas chromatography/mass spectrometry (GC/MS) 1. *Journal of Chromatographic Science* **18**, 368-369.
- Evans, D.L.; Miller, D.M.; Jacobsen, K.L.; Bush, P.B. (1987) Modulation of immune responses in mice by *d*-limonene. *Journal of Toxicology and Environmental Health* **20** (1-2), 51-66.
- Forestry Canada (1989 and previous editions) *Forest Insect and Disease Conditions in Canada*. Furniss, M.M.; Young, J.W.; McGregor, M.D.; Livingston, R.L.; Hamel, D.R. (1977) Effectiveness of controlled-release formulations of MCH for preventing Douglas-fir beetle (Coleoptera: Scolytidae) infestation in felled trees. *Canadian Entomology* **106**, 1063-1069.

- Gambliel, H.A.; Cates, R.G.; Caffey-Moquin, M.K.; Paine, T.D. (1985) Variation in the chemistry of loblolly pine in relation to infection by the blue-stain fungus. In *Proceedings of Symposium on Integrated Pest Management, April 15-18, 1985. Forest Service General Technical Report SO-56*. US Forest Service, Southern Forest Experiment Station. pp. 177-184.
- Gibbon, G.H.; Pirt, S.J. (1971) Degradation of *alpha*-pinene by *Pseudomonas* PX-1. *FEBS [Federation of European Biochemical Societies] Letters* **18**, 103-105.
- Gibson, D.E.; Moore, G.P.; Pfaff, J.A. (1989) Camphor ingestion. *American Journal of Emergency Medicine* **7**, 41-43.
- Gray, D.R.; Borden, J.H. (1989) Containment and concentration of mountain pine beetle (Coleoptera: Scolytidae) infestations with semiochemicals: Validation by sampling of baited and surrounding zones. *Journal of Economic Entomology* **82**, 1399-1405.
- Griffiths, E.T.; Bociek, S.M.; Harries, P.C.; Jeffcoat, R.; Sissons, D.J.; Trudgill, P.W. (1987) Bacterial metabolism of *alpha*-pinene: Pathway from *alpha*-pinene oxide to acyclic metabolites in *Nocardia* sp. strain, P18.3. *Journal of Bacteriology* **169**, 4972-4972.
- Hedin, P.A.; Gueldner, R.C.; Thompson, A.C. (1976) Utilization of the boll weevil pheromone for insect control. In *Pest Management with Insect Sex Attractants and Other Behavior-Controlling Chemicals*. M. Beroza (Ed). ACS Symposium Series 23. Washington, DC: American Chemical Society. pp. 30-52.
- Hillis, W.E. (1987) *Heartwood and tree exudates*. Berlin: Springer-Verlag. 268 pp.
- Hunt, D.W.A.; Borden, J.H.; Lindgren, B.A.; Gries, G. (1989) The role of autoxidation of α -pinene in the production of pheromones of *Dendroctonus ponderosae* (Coleoptera: Scolytidae). *Canadian Journal of Forest Research* **19**, 1275-1282.
- Inscoc, M.N.; Leonhardt, B.A.; Ridgway, R.L. (1990) Commercial availability of insect pheromones and other attractants. In: *Behavior-Modifying Chemicals for Insect Management: Applications of Pheromones and Other Attractants*. R.L. Ridgway, R.M. Silverstein, & M.N. Inscoc (Eds). New York: Marcel Dekker, Inc. pp. 631-715.
- Isidorov, W.A.; Zenkevich, I.G.; Ioffe, B.V. (1985) Volatile organic compounds in the atmosphere of forests. *Atmospheric Environment* **19**, 1-8.
- Le Bon, A.-M.; Cravedi, J.-P.; Tulliez, J. (1987) Fate of the isoprene hydrocarbon, pristane, in rainbow trout. *Ecotoxicology and Environmental Safety* **13**, 274-281.
- Lindgren, B.S.; McGregor, M.D.; Oakes, R.D.; Meyer, H.E. (1989a) Suppression of spruce beetle attacks by MCH released from bubble caps. *Western Journal of Applied Forestry* **4** (2), 49-52.
- Lindgren, B.S.; Borden, J.H.; Cushon, G.H.; Chong, L.J.; Higgins, C.J. (1989b) Reduction of mountain pine beetle (Coleoptera: Scolytidae) attacks by verbenone in lodgepole pine stands in British Columbia. *Canadian Journal of Forest Research* **19**, 65-68.
- Liquet, P.; Cravedi, J.-P.; Tulliez, J.; Bories, G. (1984) Growth reduction in trout induced by naphthenic and isoprenoid hydrocarbons (dodecylcyclohexane and pristane). *Ecotoxicology and Environmental Safety* **8**, 219-226.
- Manning, G.H. (1982) Impact of the mountain pine beetle on the economy of British Columbia. *BC-X - Canadian Forest Service, Pacific Forest Research Centre*. **230**, 22-23.
- McGregor, M.D.; Furniss, M.M.; Oakes, R.D.; Gibson, K.E.; Meyer, H.E. (1984) MCH pheromone for preventing Douglas-fir beetle infestation in windthrown trees. *Journal of Forest History* **82**, 613-616.
- Mitchell, E.R. (Ed). (1981) *Management of Insect Pests with Semiochemicals: Concepts and Practice*. New York: Plenum Press. 514 pp.
- Moore, R.N.; Golumbic, C.; Fisher, G.S. (1955) Autoxidation of *a*-pinene. *Journal of the American Chemical Society*, **78**, 1173-1176.
- Neeley, W.B. (1985) Environmental distribution and fate: Its role in risk assessment. In: *Toxicity Testing: New Approaches and Applications in Human Risk Assessment. Proceedings of a Meeting Held in St. Louis, MO, September 14-15, 1983*. A.P. Li (Ed). New York: Raven Press. pp. 235-246.

- Nordlund, D.A. (1981) Semiochemicals: A review of the terminology. In: *Semiochemicals: Their Role in Pest Control*. D.A. Nordlund, R.L. Jones, & W.J. Lewis (Eds). New York: John Wiley & Sons. pp. 13-28.
- Oehlschlager, A.C. (1985) Unpublished report to Phero Tech.
- Oehlschlager, A.C. (1986) *Comparison of Semiochemical Volatiles Released by Phero Tech's Mountain Pine Beetle (MPB) Tree Baits and by Trees Infested with MPB*. Unpublished report to Phero Tech.
- Opdyke, D.L.J. (1976) Monographs on fragrance raw materials, *Food and Cosmetics Toxicology* **14**, 601-633.
- Opdyke, D.L.J. (1978) Monographs on fragrance raw materials, *Food and Cosmetics Toxicology* **16** (Supplement 1), 637-886.
- Parmeggiani, L. (1987) An international viewpoint on exposure limits. *American Journal of Industrial Medicine* **14**, 213-214.
- Petersson, G. (1988) High ambient concentrations of monoterpenes in a Scandinavian pine forest. *Atmospheric Environment* **22**, 2617-2620.
- Phero Tech. (1986a) *Technical Bulletin 85003. Douglas-fir Beetle Tree Bait*.
- Phero Tech. (1986b) *Technical Bulletin 85007. Spruce Beetle Tree Bait*.
- Phero Tech. (1986c) *Research Bulletin. Western Balsam Bark Beetle - Tree Bait*.
- Phero Tech. (1987) Unpublished study on the toxicity of verbenone.
- Phero Tech. (1988) Unpublished study on emission rates of verbenone.
- Raffa, K.F.; Berryman, A.A. (1983) Physiological aspects of lodgepole pine wound responses to a fungal symbiont of the mountain pine beetle, *Dendroctonus ponderosae* (Coleoptera: Scolytidae). *The Canadian Entomologist* **115**: 723-734.
- Revised Statutes of Canada*. 1985. Chapter P-10, s. 1. *Pest Control Products Act*. Ottawa, Ontario, Canada.
- Ridgway, R.L.; Inscoc, M.N.; Dickerson, W.A. (1990) Role of the boll weevil pheromone in pest management. In: *Behavior-Modifying Chemicals for Insect Management: Applications of Pheromones and Other Attractants*. R.L. Ridgway, R.M. Silverstein, & M.N. Inscoc (Eds). New York: Marcel Dekker, Inc. pp. 437-471.
- Rudinsky, J.A. (1973) Multiple functions of the southern pine beetle pheromone verbenone. *Environmental Entomology* **2**, 511-514.
- Ryker, L.C.; Yandell, K.L. (1983) Effect of verbenone on aggregation of *Dendroctonus ponderosae* Hopkins (Coleoptera: Scolytidae) to synthetic attractant. *Zeitschrift für Angewandte Entomologie* **96**, 452-459.
- Safranyik, L.; Shrimpton, D.M.; Whitney, H.S. (1974) *Management of lodgepole pine to reduce losses from the mountain pine beetle*. Environment Canada, Forestry Service. Forestry Technical Report No. 1. 24 pp.
- Savino, J.; Tanabe, L.L. (1989) Sublethal effects of phenanthrene, nicotine, and pinane on *Daphnia pulex*. *Bulletin of Environmental Contamination and Toxicology* **42**, 778-784.
- Schwalbe, C.P. (1987) Personal communication.
- Sharratt, M. (1979) Uncertainties associated with the evaluation of the health hazards of environmental chemicals from toxicological data. In: *The Evaluation of Toxicological Data for the Protection of Public Health: Proceedings of the International Colloquium, Luxembourg, December 7-8, 1976*. New York: Pergamon Press. pp. 105-124.
- Smith, R.H. (1964) The monoterpenes of lodgepole pine oleoresin. *Phytochemistry* **3**, 259-262.
- Sower, L.L. (1987) Personal communication.
- Sower, L.L.; Overhulser, D.L.; Daterman, G.E.; Sartwell, C.; Laws, D.E.; Koerber, T.W. (1982) Control of *Eucosma sonomana* by mating disruption with synthetic sex attractant. *Journal of Economic Entomology* **75**, 315-318.
- US Code of Federal Regulations. (1990a) Title 40 Protection of Environment, Part 156—Labeling requirements for pesticides and devices, Sec. 156.10.i [Formerly Sec. 164.10] Labeling requirements; Human hazard signal word. Washington, DC: Government Printing Office.
- US Code of Federal Regulations. (1990b) Title 40 Protection of Environment, Part 158—Data Requirements for Registration.

- US Environmental Protection Agency. (1983) Exemption from FIFRA requirements for certain products containing pheromone attractants. [Supplementary information and rule.] 40 CFR Part 162—[Amended]. *Federal Register* **48**, 38572-4. [After further slight amendment, these criteria now appear in US *Code of Federal Regulations* 1990, Title 40 Protection of Environment, Part 152 —Pesticide Registration and Classification Procedures, Sec. 152.25 Exemptions for pesticides of a character not requiring FIFRA regulation.]
- US Environmental Protection Agency. (1987) Letter from A.E. Castillo to Peter J. Putland dated May 20.
- US Forest Service. (1989 and previous issues) *Forest Insect and Disease Conditions in the United States*.
- US Forest Service. (1991a) Personal communications with T.H. Hofacker (US FS), W.D. Bedard (US FS Retired), and D.L. Wood (Univ. of Calif., Berkeley) confirming an unpublished study commissioned by the US Forest Service on the toxicology of the pheromone of the western pine beetle.
- US Forest Service. (1991b) Personal communication from T.H. Hofacker (US FS) summarizing data in a 1977 report to US Forest Service from the Warf Institute on a commissioned study on the toxicology of MCH (Technical).
- US Forest Service. (1991c) Personal communication from J.A. Cota (US FS) summarizing data in a 1991 report to the US Forest Service from Wildlife International Ltd. on a commissioned study on the toxicology of MCH.
- US Forest Service. (1991d) Personal communication from T.H. Hofacker (US FS).
- US National Institute of Occupational Safety and Health. (1988) *RTECS: Registry of Toxic Effects of Chemical Substances*. Washington, DC: US Government Printing Office.
- Vanderwel, D; Oehlschlager, A.C. (1989) Pheromone biosynthesis in Coleoptera. *Chemica Scripta* **29**, 401-406.
- Yokouchi, Y.; Okaniwa, M.; Ambe, Y.; Fuwa, K. (1983) Seasonal variation of monoterpenes in the atmosphere of a pine forest. *Atmospheric Environment* **17**, 743-750.

REGULATION OF SOME CLASSES OF PHYTOCHEMICALS: FLAVOUR AND FRAGRANCE
INGREDIENTS RELATED TO INSECT BEHAVIOUR-MODIFYING CHEMICALS

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ABSTRACT

Many of the naturally occurring behaviour-modifying chemicals (BMCs) affecting insect behaviour are derived from plants. These phytochemicals have a variety of actions on insects, such as attraction to food sources, indication of suitability for oviposition, guidance of natural enemies to an area favored by their prey, or synergism with a pheromone. In addition, some of these phytochemicals are identical in structure to pheromones emitted by insects. The use of these chemicals to modify the behaviour of insects presents an opportunity to supplement existing pest control methods with chemicals of low toxicity and non-toxic modes of action. Many phytochemicals, obtained from natural sources or synthesized, are used in the flavour and fragrance industry. Consequently, considerable toxicological information has been obtained to support those uses. A review of the available toxicological data for selected groups of compounds such as acyclic terpenes, cyclic terpenoids, and aliphatic acids and esters indicates that the oral LD₅₀s in rats and the dermal LD₅₀s in rabbits are consistently greater than 1,000 mg/kg and are often greater than 5,000 mg/kg. Current use of some of these compounds ranges from hundreds to millions of kilograms/year. However, the existing regulatory structure for pesticides in the United States does not readily accommodate the toxicological data obtained for other purposes or adequately compensate for the low volume use of these relatively non-toxic compounds. The flavour and fragrance industry uses a decision-tree approach, in which existing data and experience for similar classes of compounds are examined. This approach has provided an efficient, scientific process that so far has not resulted in underestimation of toxicity. The utilization of such a decision-tree approach, in combination with the tier-testing approach provided under current US biochemical and microbial regulatory guidelines for granting waivers from data requirements for BMCs, could provide a process for reducing toxicological data requirements for insect BMCs without compromising safety considerations.

INTRODUCTION

Insect behaviour-modifying chemicals (BMCs) offer the promise of safer alternative pest control methods. It is very important to back up that promise with data illustrating the safety of these materials, so that sensible regulatory decisions may be made that will encourage the development and use of these products.

Toxicity data on lepidopteran pheromones (Inscoc & Ridgway, this volume), and coleopteran pheromones (Burke, this volume) have been reviewed. This information and related discussion provides some data and rationale that industry and government could use to make sound judgments concerning the safe use of BMCs, such as pheromones and other semiochemicals. It is also important to consider the broad range of semiochemicals of plant origin (phytochemicals, whether natural or "nature-identical"), and to take advantage of the opportunity to draw upon the

existing data which are available for these materials. With this approach, it may be possible to simplify the regulatory process without compromising safety considerations.

TOXICOLOGICAL DATA ON SELECTED FLAVOUR AND FRAGRANCE CHEMICALS

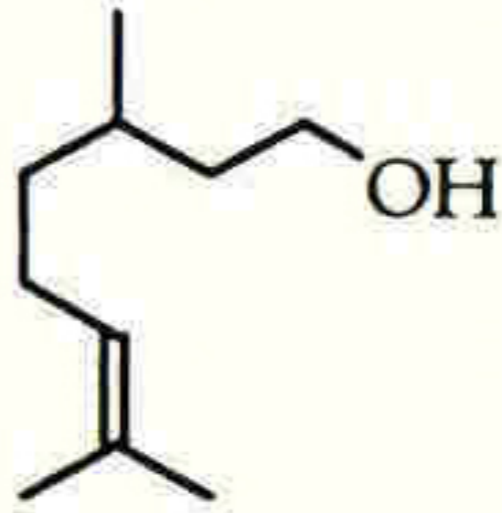
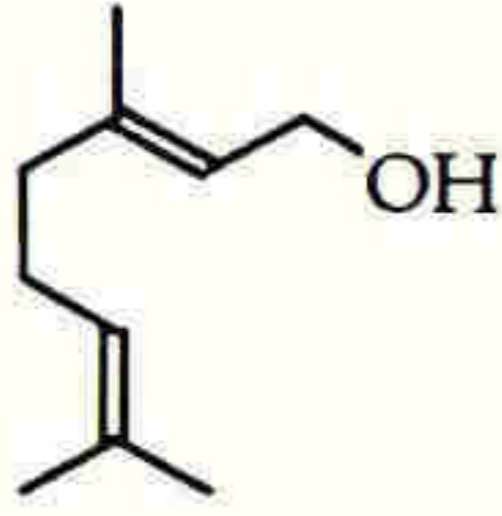
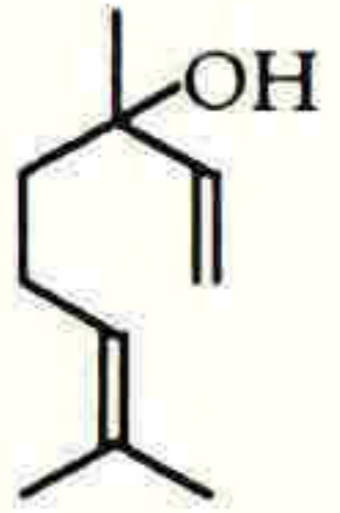
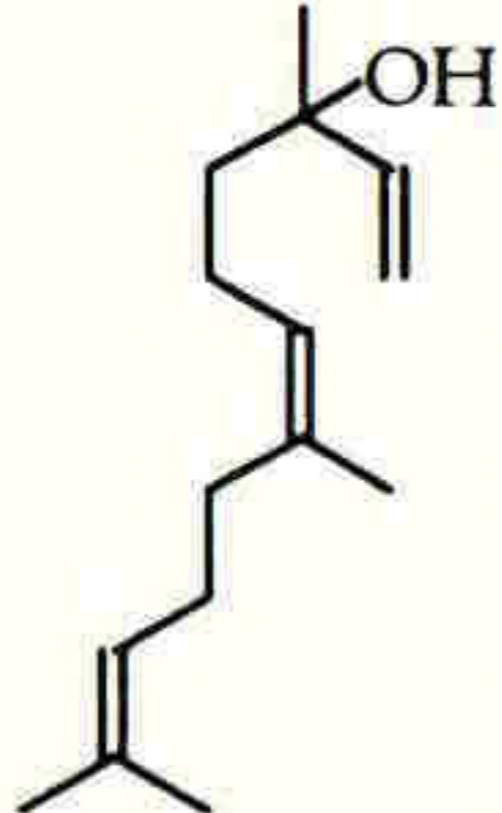
The structures of 29 chemicals commonly used by the flavour and fragrance industry are shown in tables 1-6, with some of the associated toxicological data, usage, and their relationships to semiochemicals. These chemicals are nature-identical and all but two are generally recognized as safe (GRAS) for use as food flavours. Toxicological data presented were obtained in most cases through industry funding and sharing of corporate data. The data presented in these tables were taken from data published in *Food And Chemical Toxicology*. Chemicals with additional toxicity data listed in the Registry of Toxic Effects of Chemical Substances (RTECS) (US Department of Health and Human Services, National Institute of Occupational Health and Safety) are so indicated in the tables by their RTECS numbers (US NIOSH 1991). Table 7 shows the chemical structures of some typical insect pheromones, and illustrates their similarity with the materials listed in the first six tables.

While examining the toxicological data, the definitions put forth in 1970 by the Food Protection Committee of the National Academy of Science (Food Protection Committee 1970) should be kept in mind. *Toxicity* is "the capacity of a substance to produce injury", *hazard* is "the probability that injury will result from the use of a substance in a proposed quantity and manner", and *safety* is "the practical certainty that injury will not result from the substance when used in the quantity and in the manner proposed for its use". These definitions are important to consider when evaluating the low toxicity and low exposure of many BMC-based products.

Usage considerations influenced the concentrations of flavour and fragrance compounds that were tested on human skin. Very powerful materials used in fragrances at no more than a few tenths of a percent were tested at lower levels than materials which are used at higher levels. Therefore, lower numbers obtained in irritation or sensitizing tests do not reflect higher irritating or sensitizing potential but simply indicate a known level at which they had no effect.

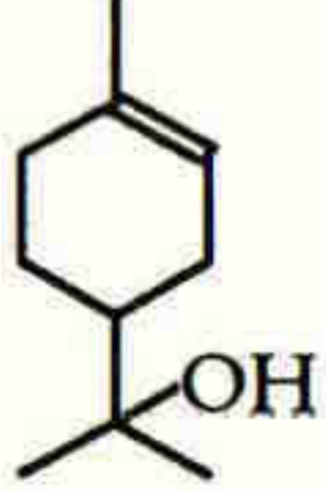
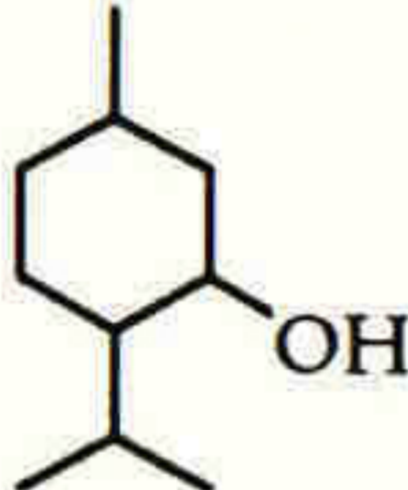
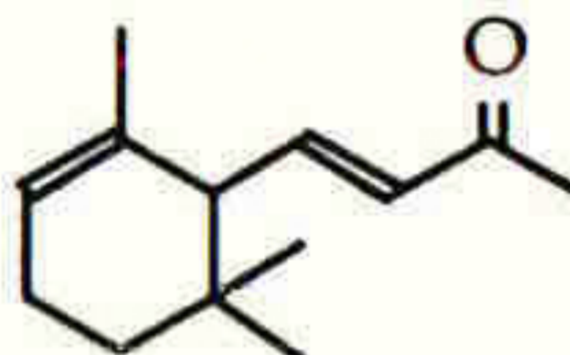
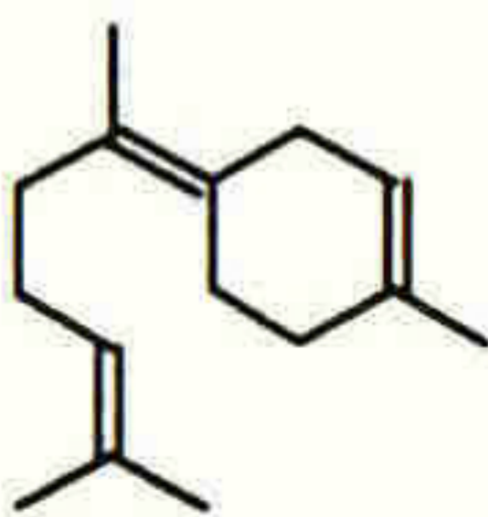
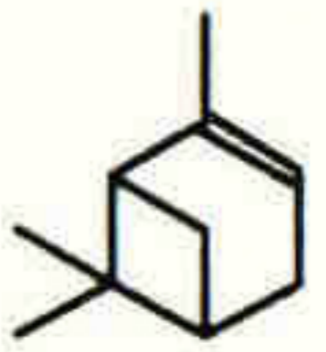
For many of the compounds in the following tables, additional toxicological data is available, including dietary intake calculations, skin absorption studies, and effects on other organisms (Cooke *et al.* 1989). The data in these tables, while representing only a portion of the total available and pertinent data, indicate that the potential hazard for many phytochemicals is low and that the use of chemical classes may be helpful in evaluating hazards.

TABLE 1. Characteristics of acyclic terpenes.

Compound	Structure	Usage in U.S. ¹	Notes ¹	Oral & Dermal Toxicity (mg/kg) ²	Non-Irritating or Sensitizing (%) ³
Citronellol (3,7-dimethyl-6-octen-1-ol)		200,000 lbs; 100,000 lbs as esters.	In use since early 1900s. Citronellol and many esters have been identified as insect behavior modifying chemicals ⁴ .	3,450; 2,650 ⁵	6 ⁵
Geraniol (3,7-dimethyl-2,6-octadien-1-ol)		1,000,000 lbs >100,000 lbs as esters	In use since early 1900s. Geraniol has been identified in over 250 essential oils. 50,000 lbs (55,000 as esters) dietary intake through foods ⁶ . Pheromone component of spider mites and a pentatomid ⁴ .	3,600; 4,800 ⁷	—
Linalool (3,7-dimethyl-1,6-octadien-6-ol)		1 million pounds. 150,000 from natural sources.	Used since 1925. Found in >10% concentration in 14 common essential oils. 150,000 lbs natural dietary intake. Both the alcohol and several esters are BMC's of several orders of insects ^{8,4} .	2,790; 5,610 ⁹	20 ⁹
Nerolidol (3,7,11-trimethyl-1,6,10-dodecatrien-3-ol)		2,000 lbs in fragrances	In use as a fragrance ingredient since the early 1900s. Pheromone component of tetranychid mites ⁴ .	>5,000; >5,000 ¹⁰	410

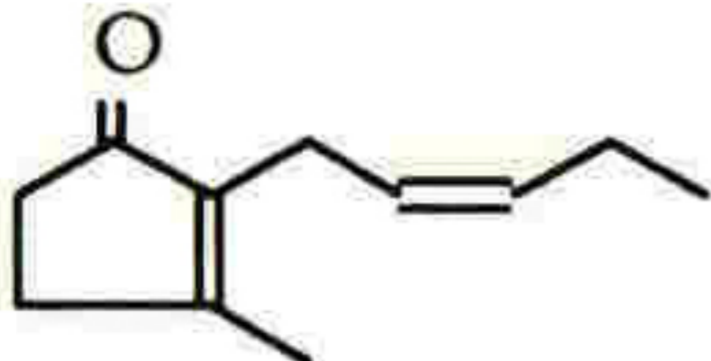
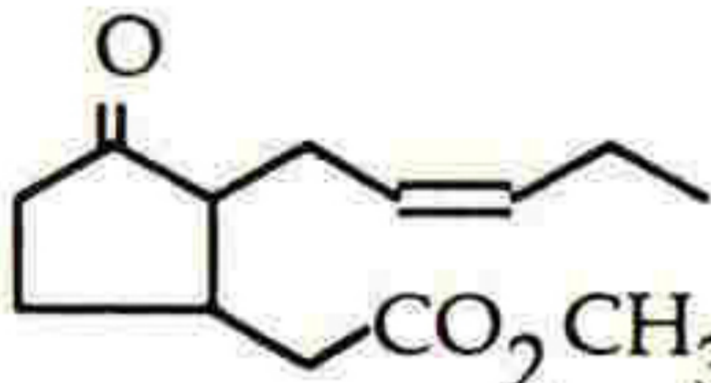
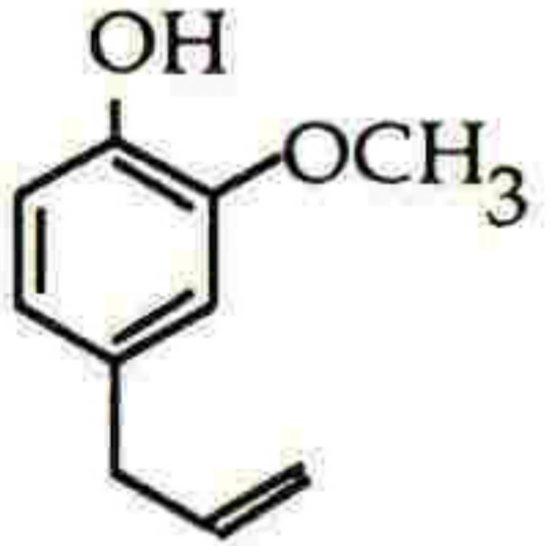

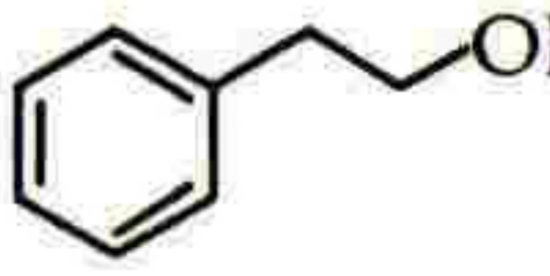
¹ Estimates, based on general knowledge of author, or from Bedoukian 1986. ² LD₅₀ values for rats and rabbits respectively. As a frame of reference, 3,450 mg/kg body weight is the equivalent of 240 grams (over 1/2 lb) for a person weighing 70 kgs (154 lbs). ³ Concentration of material having no irritating or sensitizing effects on human skin in closed 24- or 48- hour patch tests. ⁴ Mayer & McLaughlin 1991. ⁵ Opdyke 1975a, pp. 757-758; RTECS #RH3400000. ⁶ Stofberg & Grundschober 1987. ⁷ Opdyke 1974, pp. 881-882; RTECS # RG5830000. ⁸ Inscoc 1982. ⁹ Opdyke 1975a, pp. 827-832; RTECS # RG5775000. ¹⁰ Opdyke 1975a, p. 887.

TABLE 2. Cyclic Terpenoids.

Compound	Structure	Usage in U.S. ¹	Notes ¹	Oral & Dermal Toxicity (mg/kg) ¹	Non-Irritating or Sensitizing (%) ¹
<i>alpha</i> -Terpineol (<i>p</i> -menth-1-en-8-ol)		Over 1 million lbs	Identified in over 150 essential oils. The alcohol and eight esters are GRAS. A component in several pheromones and attractants ² . The acetate has been used as an attractant for the oriental fruit moth ³ .	4,300; > 3,000 ⁴	12 ⁴
l-Menthol (1-methyl-4-isopropyl cyclohexan-3-ol)		8 million lbs nat'l, 6.5 synthetic	In use before the 1900s.	3,300; >5,000 ⁵	8 ⁵
<i>alpha</i> -Ionone (2,6,6-trimethyl-2-cyclohexen-1-yl 4-(3-buten-2-one)		Over 1 million lbs of total ionones	In use since 1900. An attractant for the Malaysian Fruit Fly ⁶ .	4,590; -- ⁷	8 ⁷
Bisabolene (4-(1,5-dimethyl-4-hexenylidene)-1-methyl-1-cyclohexene)		Low usage in fragrances (200-500 lbs)	75,000 lbs dietary consumption. A component of black pepper, several isomers have been found in Isoptera ⁸ .	>5,000; >5,000 ⁹	10 ⁹
<i>alpha</i> -Pinene		>10 million lbs	Present naturally from a variety of sources. Pine trees emit pinenes and other terpenes at a rate of 100 kgs/year/hectare ¹⁰ ! 400,000 lbs dietary consumption ¹¹ . Present in many insects, part of aphid alarm pheromone ⁸ .	5,000; >5,000 ¹²	—



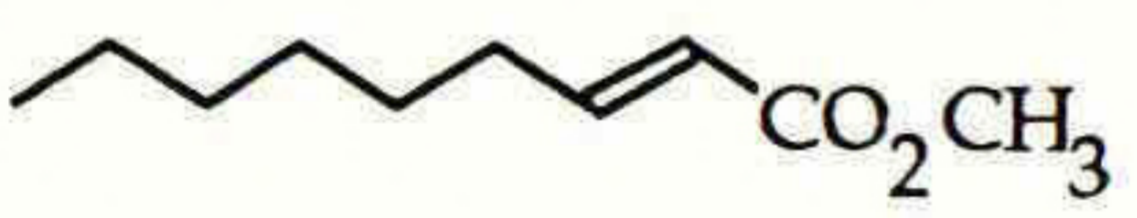


¹ See footnotes 1,2 and 3, Table 1. ² Inscoc 1982, pp. 194 & 197. ³ Mayer & McLaughlin 1991. ⁴ Opdyke 1974, pp. 997-998. ⁵ Opdyke 1976a, pp. 471-472; RTECS # OT0350000. ⁶ McGovern *et al.* 1989. ⁷ Opdyke 1975b, pp. 549-550; RTECS # NO0700000. ⁸ Morgan & Mandava 1988. ⁹ Opdyke 1975a, p. 725. ¹⁰ Lahre 1978. ¹¹ Stofberg & Grundschober 1987. ¹² Opdyke 1978, pp. 853-857.

Table 3. Miscellaneous Large Volume Aroma Chemicals.

Compound	Structure	Usage in U.S. ¹	Notes ¹	Oral & Dermal Toxicity (mg/kg) ¹	Non-Irritating or Sensitizing (%) ¹
Cis -Jasmone (3-methyl-2-(2-pentenyl)-2-cyclopenten-1-one)		ca. 2000 lbs of synthetic in fragrances.	3% in oil of jasmine. present in many other flowers, and in mint oils. Used since 1960s. Found in male lepidoptera.	5,000; >5,000 ²	8 ²
Methyl Jasmonate (Methyl (2-[(Z)-2-pentenyl])-3-oxocyclopentyl) acetate)		ca. 1000 lbs. Over 100,000 lbs of Methyl dihydrojasmonate used.	Occurs in tea, mint, and jasmine. Found in male lepidoptera. Part of a male oriental fruit moth attractant ³ .	>5,000; >5,000 ⁴ data for methyl dihydrojasmonate	20 ⁴ data for methyl dihydrojasmonate
Eugenol (4-allyl-2-methoxyphenol)		Over 100,000 lbs in fragrances.	Responsible for clove odor and taste, and present in many essential oils. 300,000 lbs natural dietary consumption ⁵ . Pheromone attractant component ³ .	2,680 ⁶ ; --	Irritant ⁶
Vanillin (4-hydroxy-3-methoxy-benzaldehyde)		1 million lbs used in F&Fs (mostly in flavours). 1000Mtons used in chocolate.	2% in vanilla beans, present in dozens of essential oils, and in wood. In use since early 1900s. Identified in insects as a pheromone and attractant ⁷ .	1,580 ⁸ ; --	28
Phenylethyl Alcohol		>10,000,000 lbs in fragrances	The alcohol and 16 of its esters are on the GRAS list. 1.5 million lbs. dietary intake. The propionate ester is an insect attractant component ³ .	1,790; 5,000 ⁹	8 ⁹






¹ See footnotes 1,2 and 3, Table 1. ² Opdyke 1979a, p. 845; RTECS # GY7301000. ³ Mayer & McLaughlin 1991. ⁴ RIFM unpublished data. ⁵ Stofberg & Grundschober 1987. ⁶ Opdyke 1975b, pp. 545-547; RTECS # SJ4375000. ⁷ Mayer & McLaughlin 1991. ⁸ Opdyke 1977, pp. 633-638; RTECS # YW5775000. ⁹ Opdyke 1975a, pp. 903-904.

TABLE 4. Aliphatic Acids and Esters.

Compound	Structure	Usage in U.S. ¹	Notes ¹	Oral & Dermal Toxicity (mg/kg) ¹	Non-Irritating or Sensitizing (%) ¹
Stearic Acid (Octadecanoic Acid)		500,000 lbs in cosmetics	Fatty acid. Used as a cosmetic component.	>15,000; >5,000 ²	7 ²
Ethyl Oleate (Ethyl (Z)-9-Octadecenoate)		—	Found in grapefruit juice and alcoholic beverages. Coleopteran pheromone component ³ .	>5,000; >5,000 ⁴	8 ⁴
Methyl 2-Nonenoate		10,000 lbs	In many fruits, and alcoholic beverages.	25,000; >5,000 ⁵	2 ⁵
Ethyl (E,Z)-2,4-Decadienoate		1000 lbs in flavours	Responsible for characteristic odor of bartlett pears. On GRAS list.	>5,000; >5,000 ⁶	3 ⁶
gamma-Dodecalactone		2000 lbs in flavours & fragrances	All aliphatic 7-12 carbon atom gamma lactones are GRAS. All found in nature in fruits and fats. Pheromone component ³ .	>5,000; >5,000 ⁷	12 ⁷






¹ See footnotes 1,2 and 3, Table 1. ² Opdyke 1979b, p. 383; RTECS # WI2800000. ³ Bestmann & Vostrowsky 1988. ⁴ Opdyke & Letizia 1982, pp. 683-685.
⁵ Opdyke 1976b, p. 811. ⁶ Ford *et al.* 1988, p. 317. ⁷ Opdyke 1976b, p. 751.

TABLE 5. Miscellaneous Aliphatics.

Compound	Structure	Usage in U.S. ¹	Notes ¹	Oral & Dermal Toxicity (mg/kg) ¹	Non-Irritating or Sensitizing (%) ¹
1,3,5-Undecatriene		500 lbs	Found in Oil of Galbanum, parsley, mango, kiwi.	>5,000; >3,000 ²	10 ²
Cetyl Alcohol (Hexadecanol)		—	Found in cheese, beef, fruit. Pheromone component.	8,400; 2,600 ³	12 ³
Leaf Alcohol (<i>cis</i> -3-Hexenol) and Acetate		100,000 lbs	Responsible for characteristic odor of green grass. Present in most fruits and vegetables. Plant volatile synergist ⁴ .	>5,000; >5,000 ⁵	—
Lauryl Acetate		200 lbs	Found in citrus products, fruits.	>5,000; >5,000 ⁶	20 ⁶
2-Undecanone		1,000 lbs	In fruits and dairy products. In use since 1900s.	>5,000; >5,000 ⁷	5 ⁷

¹ See footnotes 1,2 and 3, Table 1. ² Ford *et al.* 1988, p. 415. ³ Opdyke 1978, pp. 683-686; RTECS # MM0225000. ⁴ Dickens 1989. ⁵ Opdyke 1974, p. 909; 1975c, p. 454; RTECS # MP8400000 (*cis*-3-hexenol). ⁶ Opdyke 1976b, p. 667; RTECS # AH3525000. ⁷ Opdyke 1975a, pp. 869-870; RTECS # YQ2820000.

TABLE 6. Unsaturated Aliphatic Aldehydes.¹

Compound	Structure	Usage in U.S. ²	Notes ²	Oral & Dermal Toxicity (mg/kg) ²	Non-Irritating or Sensitizing (%) ²
(<i>E,E</i>)-2,4-Decadienal		200 lbs	In citrus, chicken and dairy products. Fatty, "chicken soup" odor.	>5,000; >1,250 ³	2 ³
(<i>Z</i>)-4-Decenal		100 lbs	Found in cooked chicken. Floral, cardamom odor.	>5,000; >5,000 ⁴	—
(<i>E,Z</i>)-2,6-Nonadienal		100 lbs	Responsible for melon and cucumber odors. Found in violet flowers. Violet, cucumber odor.	>5,000; >5,000 ⁵	2 ⁵
(<i>E</i>)-2-Tridecenal		500 lbs	Found in meats, coriander seed. Waxy, tangerine odor.	>5,000 ⁶ ; —	4 ⁶
(<i>Z</i>)-6-Nonenal		100 lbs	Found in melon and cucumber ⁷ . Very powerful melon odor.	>5,000; >5,000 ⁸	18

¹Because of their powerful odors, the aldehydes shown in the table are used in small quantities in flavours and fragrances, typically 0.1% in the formulation. ² See footnotes 1, 2 and 3, Table 1. ³ Opdyke 1979b, pp. 383-388. ⁴ Opdyke & Letizia 1982, p. 663. ⁵ Opdyke & Letizia 1982, p. 769. ⁶ Ford *et al.* 1988, p. 411. ⁷(*Z*)-6-Nonenol is present along with the aldehyde in melon and cucumber, and is a component of the olive fruit fly pheromone (Mayer & McLaughlin, 1991). ⁸ Opdyke & Letizia 1982, p. 777.

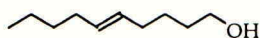
TABLE 7. Structures of Representative Pheromone Components¹.



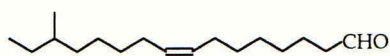
(*E,E*)-8,10-Dodecadienol
Codling moth (*Cydia pomonella*)



(*Z*)-8-Dodecenyl acetate
Oriental fruit moth (*Grapholita molesta*)



(*E*)-5-Decen-1-ol
Peach twigborer (*Anarsia lineatella*)



14-Methyl-(*Z*)-8-Hexadecenal
Khapra beetle, Warehouse beetle
(*Trogoderma granarium*, *T. variabile*)



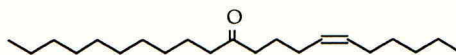
(*Z,E*)-9,12-Tetradecadienyl acetate
Lesser cornstalk borer (*Elasmopalpus lignosellus*)



(*Z*)-11-Hexadecenal
Cotton bollworm (*Helicoverpa armigera*)



(*Z,Z&Z,E*)-7,11-Hexadecadienyl acetate
Pink bollworm (*Pectinophora gossypiella*)



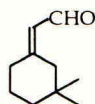
(*Z*)-6-Heneicosen-11-one
Douglas fir tussock moth
(*Orgyia pseudotsugata*)



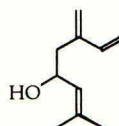
(*E*)-4-Tridecenyl acetate
Tomato pinworm
(*Keiferia lycopersicella*)



(*Z*)-9-Tricosene
Housefly (*Musca domestica*)



(*Z&E*)-3,3-Dimethyl cyclohexylidene acetaldehyde
Boll weevil (*Anthonomus grandis*)



2-Methyl-6-methylene-2,7-octen-4-ol
Bark beetles (*Ips paraconfusus*, *Ips pini*)

¹Mayer & McLaughlin 1991.

REGULATORY STATUS OF SELECTED FLAVOUR AND FRAGRANCE CHEMICALS

Status

Because many flavour and fragrance ingredients have potential uses as BMCs, including insect attractants, repellents, and pheromones, it is useful to examine the manner in which the safety of these chemicals has been assured.

The flavour and fragrance industry regularly uses over 2000 raw materials. The majority are synthetic, and like BMCs, they are mostly "nature-identical". This is not surprising, as our olfactory systems are attuned to the scents of flowers, fruits and other foods. (Fragrances typically contain from twenty to several hundred ingredients. A typical "artificial" flavour may contain from ten to several hundred ingredients, a natural flavour from several hundred to one thousand). The industry, greatly concerned with product safety issues, has taken steps to cooperatively collect toxicological data and to work closely with regulatory agencies in assessing hazard.

In the 1958 amendment to the Federal Food, Drug and Cosmetic Act, administered by the US Food and Drug Administration (FDA), the "generally recognized as safe" (GRAS) concept was developed in the law to mean "generally recognized, among experts qualified by scientific training and experience to evaluate the safety, as having been adequately shown through scientific procedures... to be safe under the conditions of intended use." (Ford 1989). An independent panel comprised of these qualified experts is today supported by the Flavour and Extract Manufacturers' Association (FEMA). They have compiled a list of over 1700 GRAS flavouring substances (Ford 1989) officially recognized by the FDA.

In 1966, the fragrance industry organized and sponsored a scientific organization, the Research Institute for Fragrance Materials (RIFM) to gather and analyze scientific data for the purpose of insuring the safety of end-product fragrances (Opdyke 1984). RIFM has sponsored studies and gathered data on 1400 common ingredients used by the industry as well as on other related materials. RIFM's use of a decision-tree approach (Cramer *et al.* 1978) and expert panels is well regarded by the FDA. It is important to note that as circumstances warrant, existing materials are re-evaluated by the industry. The International Fragrance Association (IFRA) based in Europe, collaborates closely with RIFM and, on the basis of RIFM's results, makes recommendations about the good manufacturing practice for usage of fragrance ingredients.

Process

Cramer *et al.* (1978) state that "it is neither possible nor sensible to obtain the information needed to assess every imaginable toxic risk associated with every single substance". Individual toxicologists deal with this problem by using their personal "experience" involving structure-activity relationships, metabolic mechanisms, chemical reactivity, human exposure and other relevant information. The decision-tree approach used by the US flavour and fragrance industry is designed to make the risk determination process "rational, public and explicit" (Cramer *et al.* 1978). Using currently available data on metabolism and toxicity to validate the procedure, "yes" or "no" answers to 33 questions based on chemical structure lead down branches of the tree organized by major chemical classifications, and ultimately to toxicity classifications reflecting low, moderate or serious presumed toxicity. The tree, intended for use by individuals with chemical or biochemical training, can be applied to all ingested organic and organometallic substances. This decision-tree approach for dealing with the estimation of toxic hazard (Cramer *et al.* 1978; Ford 1984) has been applied to a large number of pesticides, drugs, food additives and industrial and environmental chemicals of known biological properties, and so far has not resulted in any underestimation of

toxicity. It is important to note that under the decision-tree approach many BMCs would be classified as having low toxicity, which the specific data on the chemicals listed in Tables 1-6 serve to corroborate. The approach appears to provide a practical means for discriminating effectively among different levels of possible hazard. The estimate of possible hazard when combined with exposure based on usage data provides a very effective method for setting priorities to determine the level of data required for safety evaluation. (Along these lines, Easterday *et al.* (1984) have proposed a three-method ranking system for setting priorities for safety/risk evaluation of food ingredients based on Consumption Ratio (ratio of consumption of uncontrolled natural occurrence in food to intentional use in food), structure activity relationships (based on a decision-tree approach dealing with chemical structure) and computerized test data weighted methods.)

An additional integral part of self-regulation by the flavour and fragrance industry is the Expert Panel, independent from industry, and qualified by experience and training to make judgments on the safety of ingredients (Ford 1986). At least some of the phytochemicals used as semiochemicals or BMCs belong to chemical classes very similar to those used as flavour and fragrance ingredients, and experts are available who could be of assistance in making assessments of the safety of the materials. Exposure data will be needed to adequately assess the safety of these materials.

RELATIONSHIP BETWEEN FLAVOUR AND FRAGRANCE CHEMICALS AND INSECT BEHAVIOUR-MODIFYING CHEMICALS

Source, chemistry, and hazards

It is clear that a very close relationship and substantial overlap exist between nature-identical chemicals used as flavour and fragrance ingredients and as semiochemicals. The database generated by FEMA and RIFM is relevant to the regulation of BMCs not only because the chemical classes involved are often the same or similar, but because a large number of chemicals are actually common to both. Many male-produced pheromones are well-known flavour and fragrance chemicals, and the list of chemicals found in Hymenoptera and Isoptera, especially, includes dozens of chemicals which are also flavour and fragrance ingredients (Morgan & Mandava 1988). This is not surprising since many chemicals emitted by flowers have insect behaviour-modifying properties, and may also be a part of the insect's diet. Many other examples exist in Lepidoptera and Coleoptera (Mayer & McLaughlin 1991), and a number of flavour and fragrance chemicals are listed in tables of insect feeding deterrents or animal-produced repellents (Morgan & Mandava 1990).

The mode of action of the BMCs we are discussing is "olfactory", not toxic -- the fact that certain "smells" can be used to modify the behaviour of insects does not render the chemicals any more hazardous than when they are used in perfumes or when they occur in flowers or foods. The high structure-"odor" specificity in the responses of insects to certain BMCs should not be a cause for concern. Evaluation of the odors of the aldehydes shown in Table 6 illustrates that slight changes in chemical structure can cause significant differences in the olfactory response of humans. The same is true for semiochemicals and insects.

Regulatory implications

BMCs used for insect control are subject to regulation as pesticides. In the US, that regulation is governed primarily by the Federal Insecticide, Fungicide, and Rodenticide Act (FIFRA), administered by the US Environmental Protection Agency (EPA). This results in some

inconsistencies in the regulation of phytochemicals that may already be in use as flavour or fragrance ingredients for which the chemicals are already recognized by the FDA. Usage of phytochemicals as BMCs for insect control usually calls for far less human contact or environmental exposure than employment as ingredients in flavours or fragrances, and in many cases natural consumption of these chemicals in foods (Stofberg & Grundschober 1987) is orders of magnitude greater than potential usage as BMCs. The time and expense required to satisfy the current pesticide regulations for phytochemicals may not be economically justifiable in view of the very small comparative sales volume. In brief, the regulation of these chemicals based on "commercial intent" as "pesticides" places the most stringent regulatory burden on a low-exposure use of relatively non-toxic chemicals that is beneficial to agricultural and environmental interests. This approach does not appear to be in the best interests of society, when the increased use of these chemicals is likely to be beneficial.

An indeterminate number of phytochemicals may find use as semiochemicals for insect control. We can expect to identify more useful materials and combinations of materials at an ever increasing rate. It is not practical to require the same testing of a chemical or a natural essential oil regardless of potential exposure data, volume of use, or prior experience with the material.

CONCLUSIONS

The potential volume of insect semiochemicals required for insect trapping or control is very low, the mode of entry into the environment is usually indirect through controlled-release systems, and the classes of chemicals involved are generally of very low toxicity. Using the definitions provided by the Food Protection Committee of the National Academy of Science (Cramer *et al.* 1978) cited earlier, it is clear that the majority of semiochemical applications are of low hazard and have a large safety factor.

Phytochemicals, whether man-made or derived from nature are structurally identical ("nature-identical", a concept used in some European countries in flavours regulation), with a nature-identical mode of action (odor). Many naturally occurring chemicals already in use by industries such as the flavour and fragrance industry will find use as BMCs. However, there is limited commercial incentive to register these for minor uses as "pesticides", although there may certainly be an ecological incentive to do so. Reductions in some toxicological data requirements for semiochemicals is particularly justifiable, since they offer low hazard. Priorities and limits for toxicological data requirements must somehow be set.

An expert panel using a decision tree approach including potential exposure and chemical class information, and backed by existing toxicological and safety data, could provide assistance to the regulatory agencies in making determinations as to the appropriateness of tests required for use in insect control. Perhaps limited exemptions could be granted for semiochemicals which have already undergone evaluation for food or cosmetic use. At present, to use a natural food ingredient in a controlled-release device as a BMC or even in low concentrations as a preservative, one must go through an unduly stringent regulatory process.

The data and approach which have worked well for the flavour and fragrance industry should prove useful in facilitating the regulatory process for insect behaviour modifying chemicals.

ACKNOWLEDGMENTS

I would like to thank Dr. Richard Ford of RIFM (Englewood Cliffs, New Jersey) who took the time to discuss this topic with me, and who authored or co-authored many of the references cited.

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REFERENCES

- Bedoukian, P.Z. (1986) *Perfumery and Flavouring Synthetics*, third edition. Wheaton, IL: Allured Publishing Corporation.
- Bestmann, H.J., Vostrowsky, O. (1988) Pheromones of the Coleoptera. In: *Handbook of Natural Pesticides*, Vol. IV, Pt. A., E.D. Morgan & N.B. Mandava (Eds), Boca Raton, FL: CRC Press, pp. 95-183.
- Cooke, M.A.; Cadby, P.A.; Ford, R.A. (1989) Databases in the Perfumery Industry. *Seminars in Dermatology*, 8 (2), 85-87.
- Cramer, G.M.; Ford, R.A.; Hall, R.L. (1978) Estimation of Toxic Hazard -- A Decision Tree Approach. *Food and Cosmetics Toxicology* 16, 255-276.
- Dickens, J.C. (1989) Green leaf volatiles enhance aggregation pheromone of boll weevil, *Anthonomus grandis*. *Entomologia Experimentalis et Applicata* 52, 191-203.
- Easterday, O.D.; Hall, R.L.; Ford, R.A.; Rulis, A.M. (1984) A Combined Three-Method Safety/Risk Priority Ranking System. Hommage au Professeur Rene Truhaut, Imprimerie Tardy Quercy (S.A.). pp. 338-342.
- Food Protection Committee (1970) *Evaluating the Safety of Food Chemicals*. National Academy of Science/National Research Council, Washington D.C.
- Ford, R.A. (1984) Setting Priorities for Testing Essential Oils. *Journal of Applied Cosmetology*, 2, 28-34.
- Ford, R.A. (1986) The Function of Expert Panels in Evaluating the Safety of Flavouring and Fragrance Materials. Presented at the 10th International Congress of Essential Oils, Fragrances & Flavours, Washington D.C. November 16-20, 1986.
- Ford, R.A. (1989) General Principles of the Regulation of Flavours. In: *International Food Regulation Handbook*, R.D. Middlekauff & P. Shubik (Eds), New York: Marcel Dekker, pp. 241-252.
- Ford, R.A.; Letizia, C.; Api, A.M. (1988) Fragrance Raw Materials Monographs, *Food and Chemical Toxicology*, 26, 273-415.
- Inscoc, M.N. (1982) Insect Attractants, Attractant Pheromones, and Related Compounds. In: *Insect Suppression with Controlled Release Pheromone Systems*. Kydonieus, A. F. & Beroza, M. (Eds.) Vol. II, Boca Raton, FL: CRC Press, pp. 201-295.
- Lahre, T. (1978) Emissions from vegetation, cutback asphalts, bakeries, oil shale, natural gas leaks, and urban solvent evaporation. *Conference on Emission Factors and Inventories*, November 13-16, Anaheim, CA, Air Pollution Control Association (Eds.), pp. 18-25.
- Mayer, M.S.; McLaughlin, J.R. (1991) *Handbook of Insect Pheromones and Sex Attractants*, Boca Raton, FL: CRC Press.
- McGovern, T.P.; Flath, R.A.; Cunningham, R.T. (1989) Attractants for *Dacus latifrons*, the Malaysian fruit fly. US Patent 4,877,607.
- Morgan, E.D.; Mandava, N.B. (1988) *Handbook of Natural Pesticides*. Vol. IV. Pheromones Part B. Boca Raton, FL: CRC Press. 291 pp.
- Morgan, E.D., Mandava, N.B., (1990) *Handbook of Natural Pesticides*. Vol. VI. Insect Attractants and Repellents, Boca Raton, FL: CRC Press. 249 pp.

- Opdyke, D.L.J. (1974) Monographs on fragrance raw materials. *Food and Cosmetics Toxicology*, 12 (Supplement), 827-998.
- Opdyke, D.L.J. (1975a) Monographs on fragrance raw materials. *Food and Cosmetics Toxicology*, 13 (Supplement), 681-923.
- Opdyke, D.L.J. (1975b) Monographs on fragrance raw materials. *Food and Cosmetics Toxicology*, 13, 545-554.
- Opdyke, D.L.J. (1975c) Monographs on fragrance raw materials. *Food and Cosmetics Toxicology*, 13, 449-457.
- Opdyke, D.L.J. (1976a) Monographs on fragrance raw materials. *Food and Cosmetics Toxicology*, 14, 443-481.
- Opdyke, D.L.J. (1976b) Monographs on fragrance raw materials. *Food and Cosmetics Toxicology*, 14, (Supplement), 665-893.
- Opdyke, D.L.J. (1977) Monographs on fragrance raw materials, *Food and Cosmetics Toxicology*, 15, 611-638.
- Opdyke, D.L.J. (1978) Monographs on fragrance raw materials, *Food and Cosmetics Toxicology*, 16 (Supplement 1), 637-886.
- Opdyke, D.L.J. (1979a) Monographs on fragrance raw materials, *Food and Cosmetics Toxicology*, 17 (supplement), 695-923.
- Opdyke, D.L.J. (1979b) Monographs on fragrance raw materials, *Food and Cosmetics Toxicology*, 17, 357-390.
- Opdyke, D.L. (1984) The RIFM Story. In: *The Cosmetic Industry - Scientific and Regulatory Foundations*, Estrin, N.F. (Ed), Marcel Dekker. New York, pp. 231-243.
- Opdyke, D.L.J., Letizia, C. (1982) Monographs on fragrance raw materials, *Food and Chemical Toxicology*, 20 (supplement), 683-685.
- Stofberg, J., Grundschober, F. (1987) Consumption Ratio and Food Predominance of Flavouring Materials, Third Cumulative Series, *Perfumer & Flavourist*, 12, pp. 27-68.
- US National Institute of Occupational Health and Safety (US NIOSH) (1991) *Registry of Toxic Effects of Chemical Substances*. Washington, DC: US Government Printing Office

EXPOSURE, FATE AND POTENTIAL RESIDUES IN FOOD OF APPLIED LEPIDOPTERAN PHEROMONES

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ABSTRACT

Chemical pesticide registration regulations in most of the world require extensive toxicity, environmental and residue testing of proposed active ingredients. These definitions currently include insect pheromone components used to disrupt or confuse mating cycles. Negligible residues are predicted for lepidopteran pheromones used in fruit production as only small amounts are employed, and in discrete point source formulations applied in use patterns that preclude direct contact with the fruit. In this study, fruits (apples, peaches, grapes) treated with a variety of pheromones were analyzed for their respective component residues. Fruit samples were blended and extracted with acetone; following the addition of water, the analytes were extracted into hexane, concentrated, and adsorbed onto a Florisil Sep-pak. Elution was with 10% acetone/hexane. Chromatography of Z-9-DDA (Z-9-Dodecen-1-ol Acetate), Z-11-TDA (Z-11-Tetradecen-1-ol Acetate) and E-11-TDA (E-11-Tetradecen-1-ol Acetate) utilized a H-P Model 5890 equipped with a Restek Stabilwax 10 capillary column, 30 m x 0.25 mm x 0.25 µm coating. Temperature program: 80°-130°C @ 5°/min, 130°-200°C @ 4°/min, hold 9 min. Detection by HP-MSD Model 5970B was in the selective ion mode. Retention times were 16.3, 21.1 and 20.9 min, respectively. EZ-3,13-ODA (E-Z-3,13-Octadecadien-1-ol Acetate) and ZZ-3,13-ODA (Z-Z-3,13-Octadecadien-1-ol Acetate) were chromatographed on a H-P Model 5890B using a Silar 10C, 50m x 0.25 mm x 0.25 µm column. Temperature program: initial temperature 80°C, hold 2 min.; 80°-130°C @ 10°C/min., hold 15 min. Detection was by HP-MSD Model 5970C operated in the selective ion mode. Retention times were 18.4 and 18.6 min., respectively. Recoveries were generally 80%, or better, at a minimum sensitivity of <5 ppb for all components analyzed. No residues have been detected on any commodity samples.

INTRODUCTION

The large number of insect pheromones being evaluated or proposed for formal submission and registration as commercial pest control agents poses a problem since in most countries current regulations require that each component in the formulation be subjected to the same testing and toxicity standards as are all potential chemical pesticide active ingredients. This is prohibitively expensive, both because of the large number of active ingredients needed, and because of the relatively small market potential for each specific pheromone mixture. Not only are pheromone compositions unique for a given target species, they are applied at lower rates than traditional insecticides (gram/hectare vs. kilogram/hectare). Couple these factors with the separate registration and tolerance

required for each commodity on which a given pheromone component may be used, and the commercial future for this whole area of endeavor is bleak. Clearly, each step of the traditional chemical pesticide regulatory process must be examined with regard to the use patterns and chemical characteristics of the proposed pheromone components so that those procedures that could be modified, minimized or combined without compromising the safety and intent of the law might be so amended. This paper considers pheromone residues on food, an area that must be addressed in present chemical pesticide registration protocols.

Previous work at our laboratory had shown no detectable residues (<2 ppb) of either Z-9-DDA or Z-11-TDA on grapes (see Table I) after season-long application programs as high as 141 g/ha (Spittler et al., 1988). However, there is little other available literature on pheromone residues in and on raw agricultural commodities: obviously this one report constitutes insufficient evidence for consideration of regulatory relief from residue studies. Accordingly, we acquired treated fruit samples from several 1990 lepidopteran pheromone field trials for residue analysis--the intent being to expand the pheromone residue data base. The similarity of many components in commercial lepidopteran mating disruption formulations simplified the analytical complexity of the study (Figure 1). Our preliminary attempts to chromatograph the free alcohols were discouraging, but agreed with previous observations of problems with quantitative measurements of underivatized alcohol pheromone components (Charlton, personal communications). However, if conversion of the alcohols to their respective acetates could be effected quantitatively and with acceptable recoveries, five of the eight alcohols could be determined as analytes already present in the scheme. Only data for the acetate pheromone components are available for this presentation. Measurement of the alcohol pheromone component residues will be conducted and reported in future work.

PRODUCTION OF FIELD SAMPLES

All field trials except the New York State grape berry moth studies were part of the 1990 field testing program of Biocontrol Ltd., Davis, CA 95616 USA. All pheromones tested were formulated in polyethylene twist-tie dispensers at load rates described in this paper. Formulations were prepared for Biocontrol Ltd. by Shin-Etsu Chemical Company, Tokyo, Japan. Species information on four of the insect pheromones being assessed is proprietary and confidential. Thus, these insects have been code named 9OUSEX1, 9OUSEX2, 9OUSEX3 and 9OUSEX4 in this publication to protect trade information. See Tables for full names and percentages of pheromone components.

Grapes, New York

Details on the 1986 program are found in Spittler et al., 1988. For T. J. Dennehy's 1990 trials, vineyards of Seyval and Elvira varieties were hung with Grape Berry Moth pheromone in tie-on polyethylene applicators at a rate of 493 ties/ha on April 15, 1990. Each tie contained 69 mg of a mixture of 90% Z-9-DDA and 10% Z-11-TDA; total application rate 34 g/ha. Fresh samples were delivered within several hours of harvest, on September 20, 1990, to the Cornell Analytical Laboratories. Samples were stored at -20°C until analysis. See Tables 1 and 2.

Grapes, Virginia

Two Chardonnay vineyards were hung with 493 Grape Berry Moth ties/ha (see above)--Prince Michel on April 21, 1990 and Meredith on April 22, 1990--by D. Pfeiffer. On August 30, 1990, samples were taken from row #15 (center row) and row

#6 of the Prince Michel vineyard. On September 9, 1990, a sample was taken from the center row of Meredith vineyard. Samples were frozen for shipping, and held at -20°C upon receipt. See Table 3.

Apples, Virginia

One orchard (Bryant) of Golden Delicious variety was treated on April 12, 1990 by D. Pfeiffer with 986 ties/ha of Codling Moth pheromone (63% EE-8,10-DDOH, 31% DDOH, 6% TDOH). Each tie contained 170 mg of formulation; total application 168 g/ha. Both the Bryant orchard and a Wine Sap variety orchard (Crown Spring Valley) were hung on April 12-13, 1990 with 986 ties/ha of 9OUSEX4 pheromone. One hundred and thirty mg/tie yields 129 g/ha. Two replicate samples from Bryant were harvested September 4, 1990, frozen for shipping, and maintained at -20°C until analysis. One sample from Crown Spring Valley was taken October 11, 1990, and handled in a similar manner. See Tables 4 and 8.

Apples, New York

Orchard blocks of Tydeman, McIntosh, Cortland and Ida Red variety apples were hung on June 1, 1990 with 986 ties/ha of 9OUSEX1 pheromone by A. Agnello. Each tie contained 160 mg of Z-11-TDA. Harvest dates were Tydeman-August 22, 1990; McIntosh-September 17, 1990; Cortland-September 21, 1990; and Ida Red-October 5, 1990. Samples were delivered fresh to the laboratory on their harvest dates where they were pulverized, subsampled and frozen at -20°C. See Table 5.

Apples, Pennsylvania

9OUSEX2 pheromone formulations were under investigation by L. Hull. "Generic" ties contained 67% E-11-TDA, 29% Z-11-TDA, 1% E-11-TDOH, 1% Z-11-TDOH and 2% Z-9-DDA. Two blocks (Tyson and Oyler) of Yorking variety were hung with generic ties on April 18, and 17, 1990, respectively: 1972 ties/ha x 160 mg/tie = 316 g/ha. "High E" ties contained 90% E-11-TDA and 10% E-11-TDOH. Orchards and rates were Yorking-Hall (1479 ties/ha x 160 mg/tie = 237 g/ha), Yorking-Raff and Rome-Hickey (985 ties/ha x 160 mg/tie = 158 g/ha). Harvests were on September 27, October 6, October 3, October 2, and October 15, 1990, in the order presented. Samples were frozen for shipment and maintained at -20°C until analyzed. See Table 6.

Peaches, New Jersey

Two variety blocks, Marqueen and Rio-Oso-Gem, were treated by D. Polk with 247 ties/ha of 9OUSEX3 pheromone on April 1-2, 1990. Each 50 mg tie was 70% EZ-3,13-ODA and 30% ZZ-3,13-ODA. Total application 12.5 g/ha. The Marqueen block was sampled August 21, 1990, and the Rio-Oso-Gem block on August 29, 1990. Encore variety samples were taken on this latter date as untreated check. Samples were frozen for shipping and stored at -20°C. See Table 7.

ANALYSIS

Sample Preparation

Fruit was pulverized in a Hobart chopper, either upon receipt or immediately before analysis. Fifty gram samples were taken from the homogeneous slurry. Each 50g sample was blended for two min with 2.5g Hyflo-Supercel and 60 ml redistilled acetone. Each extraction mixture was filtered in a sintered glass funnel and the resultant pad rinsed with acetone. Fifty ml H₂O, 15 ml saturated NaCl and 50 ml n-hexane were added to the

filtrate in a one-liter separatory funnel, and shaken. After phase separation, the hexane was removed and the aqueous phase was sequentially extracted with two more 50 ml portions of n-hexane--these were then combined with the original and dried over Na₂SO₄. Volume was reduced to 5.0 ml by rotovap @ 35°C. Any waxy precipitants were centrifuged out, and a 2.0 ml (20g equivalent) aliquot was placed on a Florisil Sep-pak (Waters Assoc, Milford, MA USA). After first washing with 5.0 ml of n-hexane, the pheromone containing fraction was eluted with 2.0 ml of 10%-acetone/90% n-hexane. The sample was evaporated under dry N₂ to 1.0 ml.

Standards

Analytical standards were obtained from Shin-Etsu Chemical Company, Ltd., Tokyo: Z-9-DDA, Lot #04008; Z-11-TDA, Lot #98007; EE-8,10-DDOH, Lot #03050; DDOH, Lot #03065; TDOH, Lot #03066; E-11-TDA, Lot #03066; E-11-TDOH, Lot #03037; Z-11-TDOH, Lot #83023; EZ-3,13-ODA, Lot #93006; ZZ-3,13-ODA, Lot #16280.

Chromatography: Z-9-DDA; Z-11-TDA; E-11-TDA

The samples (1.0 µl) were injected on a Hewlett-Packard Model 5890 B Capillary Gas Chromatograph utilizing a split-splitless injector at 245°C. Column was a Restek Stabilwax 10, 30m x 0.25 mm I.D. x 0.25 µm coating with a He carrier velocity of 30 cm/sec. Temperature program: initial temperature 80°C, hold 1.0 min; 80°C to 130°C @ 10°C/min; 130°C to 200°C @ 4°C/min, hold 9.0 min; 200°C to 250°C @ 30°C/min, hold/recycle. Transfer line to the H-P 5970B detector was via a butt connector/guard column maintained at 280°C.

Chromatography: EZ-3,13-ODA; ZZ-3,13-ODA

Samples (1.0 µl) were injected on a Hewlett-Packard 5890B Capillary Gas Chromatograph utilizing a split-splitless injector at 220°C. Column was Silar 10C, 50m x 0.25 mm x 0.25 µm coating with a He carrier velocity of 25 cm/sec. Temperature program: 80°C, hold 2 min.; 80°C to 220°C @ 10°C/min.; hold 15 min.; recycle. Transfer line to the H-P 5870C detector was via a butt connector/guard column maintained at 250°C.

Detection

Quantitation was with Hewlett-Packard Model 5970B or 5970C Mass Selective Detectors run in the SIM (Selective Ion Mode) at the major unique M/E (Mass/Charge Ratio) for each component (See Figures 2-9). These figures give the SIM response for the various analytes. Untreated check materials for each commodity were run at M/E's determined for the base peaks of each pheromone, as were spiked checks. For all acetates investigated, the strongest ion corresponded to M-60, the ion formed by the loss of an acetate fragment. Figures 5 and 9 illustrate samples receiving the higher application rates of selected pheromone components. See Spittler, 1988 for Z-9-DDA and Z-11-TDA analytical details on grapes.

Recovery and Sensitivity

In Table 9 are found the results of recovery spikes run for pheromone components on check samples. In those situations where no control samples of a particular variety were available, the test samples were spiked after determining the retention windows of interest to be free of interference at the designated M/E's.

Figure 1

Simplification of Leiodopteran Pheromone Analytical Scheme

PHEROMONE COMPONENTS		ANALYTES
GBM	Z-9-DDA Z-11-TDA	Z-9-DDA (2)
9OUSEX1	Z-11-TDA	Z-11-TDA (3+2*)
9OUSEX4	Z-11-TDOH* E-11-TDOH*	E-11-TDA (2+3*)
9OUSEX2 GENERIC	E-11-TDA Z-11-TDA E-11-TDOH* Z-11-TDOH* Z-9-TDA	
9OUSEX2 HIGH E	E-11-TDA E-11-TDOH*	
GM	EE-8,10-DDOH* DDOH* TDOH*	EE-8,10-DDA DDA TDA
9OUSEX3	EZ-3,13-ODA ZZ-3,13-ODA	EZ-3,13-ODA ZZ-3,13-ODA

*If Acetylated to Corresponding Acetate

TABLE 1

Residues of Grape Berry Moth Pheromone* on Grapes in New York State -- 1986.

VARIETY-SITE	RATE		DATE(month/day)		RESIDUE (ppb)	
	TIES/ha	(g/ha)	APPLICATION	HARVEST	Z-9-DDA	Z-11-TDA
CONCORD-HAYWARD	985	(86)	5/15	10/1	<5	<5
CONCORD-HAYWARD	986	(86)	5/15 & 7/15	10/1	<5	<5
CONCORD-HAYWARD	0	(0)	---	10/1	<5	<5
CONCORD-FRANCIS	986	(86)	5/15	10/1	<5	<5
CONCORD-FRANCIS	493	(43)	5/15	10/1	<5	<5
CONCORD-FRANCIS	0	(0)	---	10/1	<5	<5
CONCORD-DEGOLIER	1972	(172)	5/15 & 7/15	10/1	<5	<5
CONCORD-DEGOLIER	986	(86)	5/15	10/1	<5	<5
CONCORD-DEGOLIER	0	(0)	---	10/1	<5	<5

FROM: Spittler, Leichtweis and Dennehy, ACS, 6156/88/0379

*Grape Berry Moth Pheromone = 90% Z-9-Dodecen-1-ol Acetate (Z-9-DDA)

88 mg/TIE 10% Z-11-Tetradecen-1-ol Acetate (Z-11-TDA)

TABLE 4

Residues of Codling Moth Pheromone* on Apples in Virginia -- 1990.

VARIETY-SITE	RATE		DATE(month/day)		RESIDUE (ppb)		
	TIES/ha	(g/ha)	APPLICATION	HARVEST	EE-8,10-DDOH	DDOH	TDOH
GOLDEN DELICIOUS -BRYANT #1	986	(168)	4/12	9/4/90	Analyses Incomplete		
GOLDEN DELICIOUS -BRYANT #2	986	(168)	4/12	9/4/90			

Field Research: Pfeiffer, Virginia Polytechnical Institute

*Codling Moth Pheromone = 63% E,E-8,10-Dodecadien-1-ol (EE-8,10-DDOH)
 170 mg/TIE 31% Dodecan-1-ol (DDOH)
 6% Tetradecan-1-ol (TDOH)

TABLE 5

Residues of 9OUSEX-1 Pheromone* on Apples in New York -- 1990.

VARIETY-SITE	RATE		DATE(month/day)		RESIDUE (ppb)
	TIES/ha	(g/ha)	APPLICATION	HARVEST	Z-11-TDA**
TYDEMAN-OAKS	986	(158)	6/1	8/22	<5
TYDEMAN-OAKS	0	(0)	---	8/22	<5
MCINTOSH-PADDOCK	986	(158)	6/1	9/17	<5
MCINTOSH-PADDOCK	0	(0)	---	9/17	<5
CORTLAND-STAPLES	986	(158)	6/1	9/21	<5
CORTLAND-STAPLES	0	(0)	---	9/21	<5
IDA RED-STAPLES	986	(158)	6/1	10/5	<5
IDA RED-STAPLES	0	(0)	---	10/5	<5

Field Research: Agnello, Cornell University

*9OUSEX-1 Pheromone = 100% Z-11-Tetradecen-1-ol Acetate (Z-11-TDA)
 160 mg/TIE

**Samples run in duplicate

TABLE 6

Residues of 9OUSEX-2 Pheromone* on Apples in Pennsylvania -- 1990.

VARIETY-SITE	RATE		DATE(month/day)		RESIDUE (ppb)				
	TIES/ha	(g/ha)	APPLICATION	HARVEST	E-11-TDA	Z-11-TDA	E-11-TDOH	Z-11-TDOH	Z-9-DDA
YORKING-TYSON	1972	(316)	5/18	9/27	<5	<5			<5
YORKING-OYLER	1972	(316)	5/17	10/6	<5	<5	Analyses Incomplete		<5
YORKING-HALL	1479	(237)	5/18	10/3	<5	--	For Alcohols		--
YORKING-RAFF	985	(158)	5/15	10/2	<5	--			--
YORKING-RAFF	0	(0)	---	10/2	<5	<5			<5
ROME-HICKEY	985	(158)	7/27	10/15	<5	--			--
ROME-HICKEY	0	(0)	---	10/15	<5	--			--

Field Research: Hull, Pennsylvania State University

*9OUSEX-2 Pheromone, Generic = 160 mg/TIE
 100% E-11-Tetradecen-1-ol Acetate (E-11-TDA)
 29% Z-11-Tetradecen-1-ol Acetate (Z-11-TDA)
 1% E-11-Tetradecen-1-ol (E-11-TDOH)
 1% Z-11-Tetradecen-1-ol (Z-11-TDOH)
 2% Z-9-Dodecen-1-ol Acetate (Z-9-DDA)

*9OUSEX-2 Pheromone, High E = 160 mg/TIE
 90% E-11-Tetradecen-1-ol Acetate (E-11-TDA)
 10% E-11-Tetradecen-1-ol (E-11-TDOH)

TABLE 7

Residues of 9OUSEX-3 Pheromone* on Peaches in New Jersey -- 1990.

VARIETY-SITE	RATE		DATE(month/day)		RESIDUE (ppb)	
	TIES/ha	(g/ha)	APPLICATION	HARVEST	EZ-3,13-ODA	ZZ-3,13-ODA
MARQUEEN-RFRDC	247	(12.5)	5/1-2	8/21	<5	<5
RIO-OSO-GEM-RFRDC	247	(12.5)	5/1-2	8/29	<5	<5
ENCORE-RFRDC	0	(0)	---	8/29	<5	<5

Field Research: Polk, Rutgers University

*9OUSEX-3 Pheromone = 70% E-Z-3,13-Octadecadien-1-ol Acetate (EZ-3,13 ODA)
 50 mg/TIE 30% Z-Z-3,13-Octadecadien-1-ol Acetate (ZZ-3,13 ODA)

TABLE 8

Residues of 9OUSEX-4 Pheromone* on Apples in Virginia -- 1990.

VARIETY-SITE	RATE		DATE(month/day)		RESIDUE (ppb)	
	TIES/ha	(g/ha)	APPLICATION	HARVEST	Z-11-TDOH	E-11-TDOH
WINE SAP-CROWN SPRING VALLEY	986	(129)	4/13	10/11		
GOLDEN DELICIOUS- BRYANT #1	986	(129)	4/12	9/4	Analyses Incomplete	
GOLDEN DELICIOUS- BRYANT #2	986	(129)	4/12	9/4		

Field Research: Pfeiffer, Virginia Polytechnical Institute

*9OUSEX-4 Pheromone = 30% Z-11-Tetradecen-1-ol (Z-11-TDOH)
 130 mg/TIE 70% E-11-Tetradecen-1-ol (E-11-TDOH)

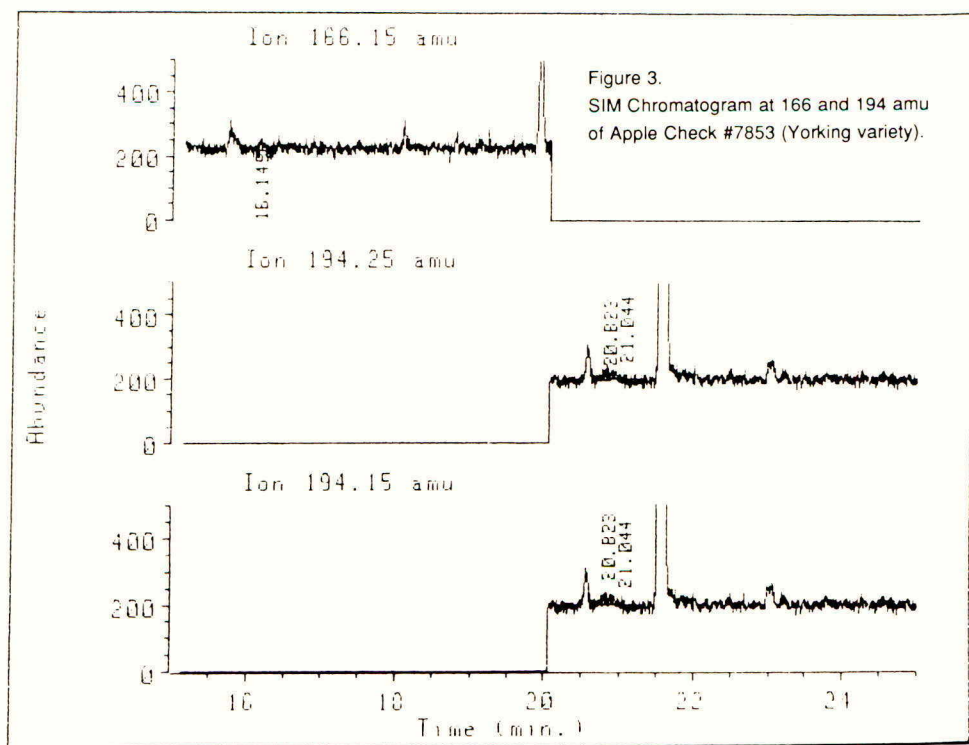
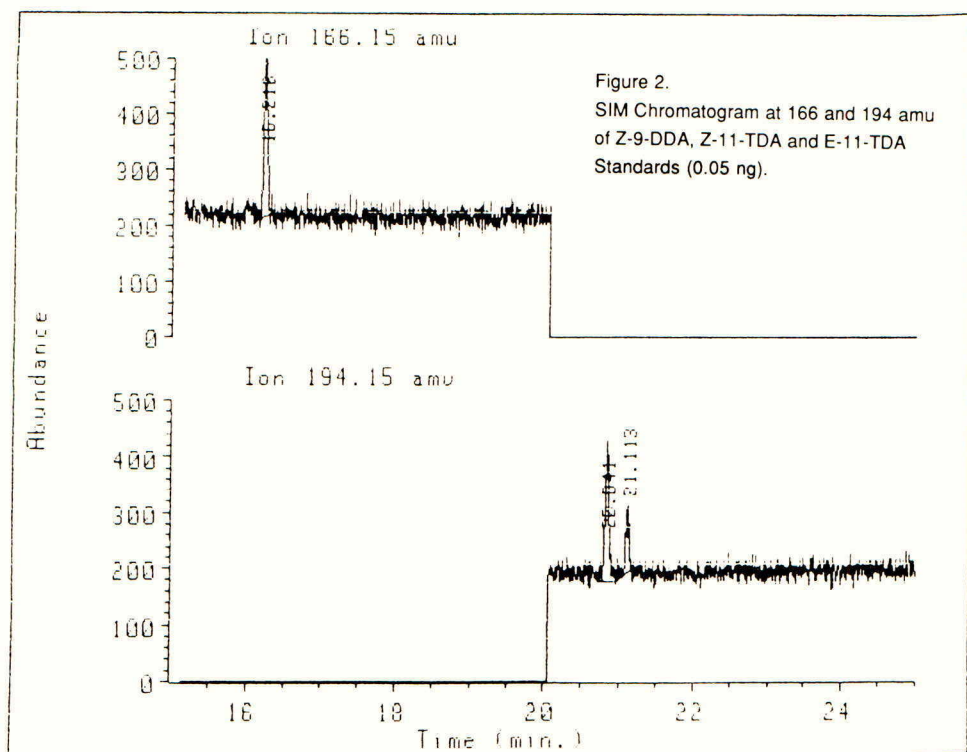
TABLE 9

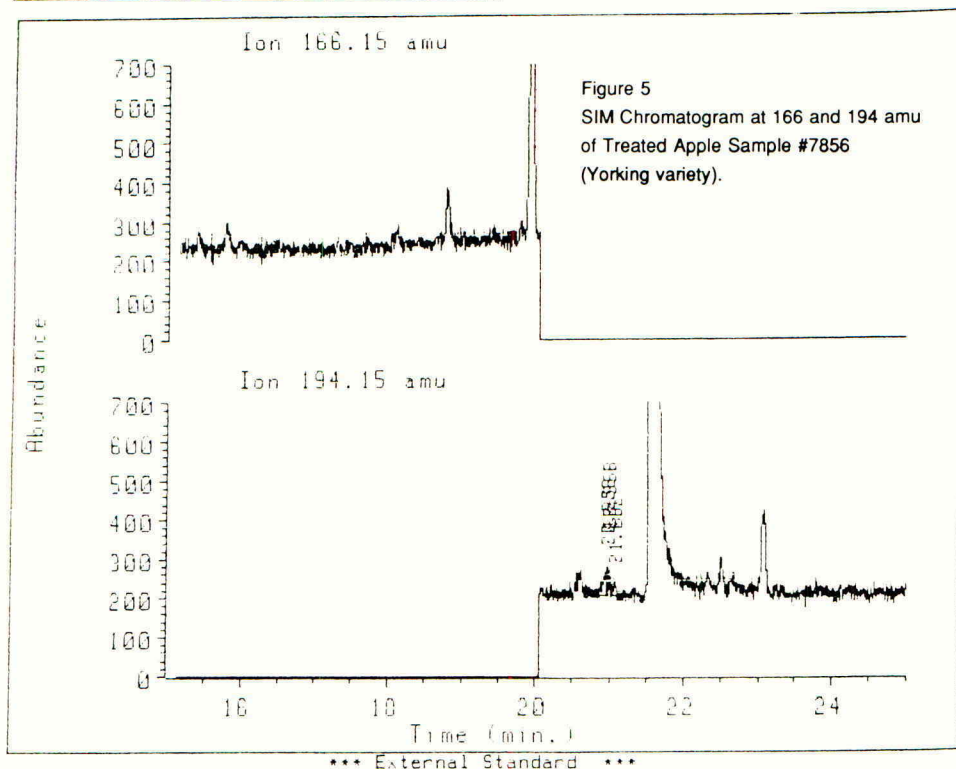
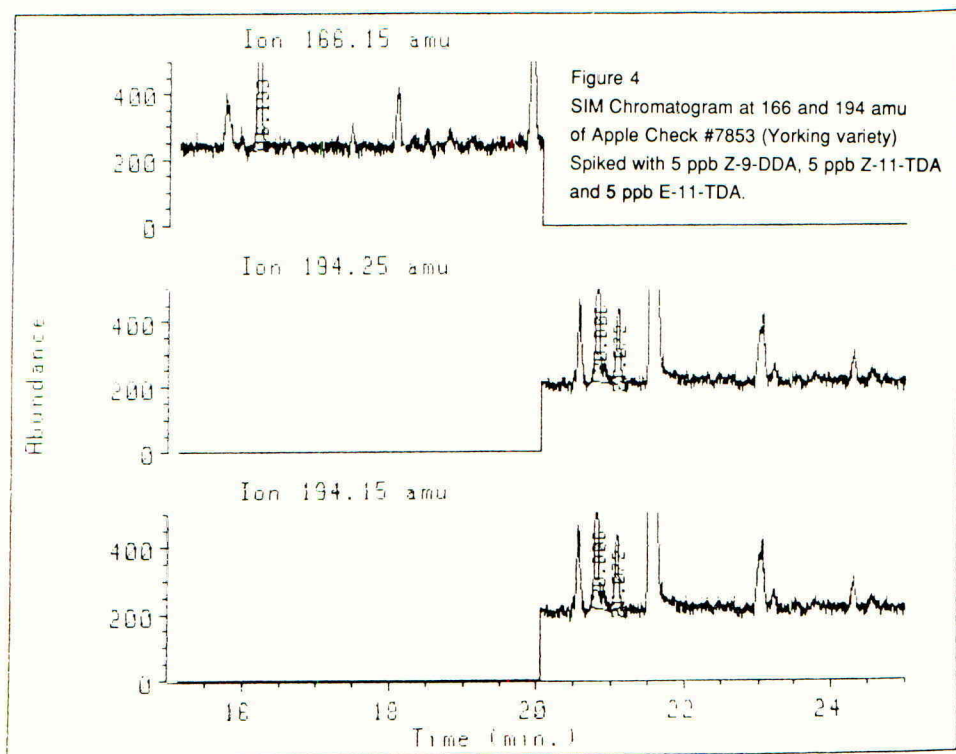
Retention Times, Recoveries and Sensitivities for Pheromone/Commodity Analytical Methods

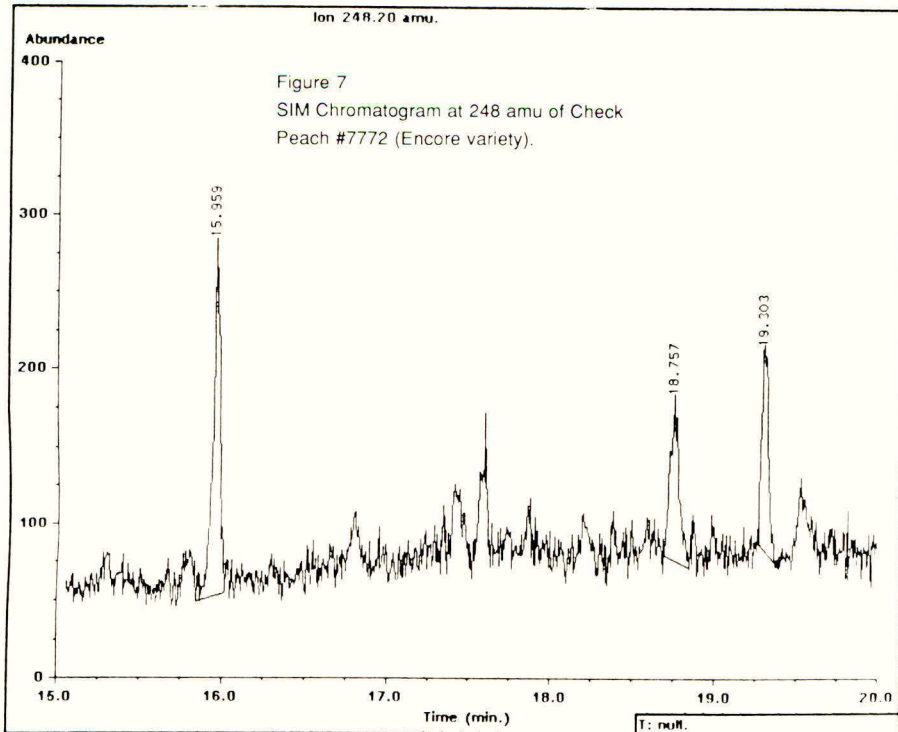
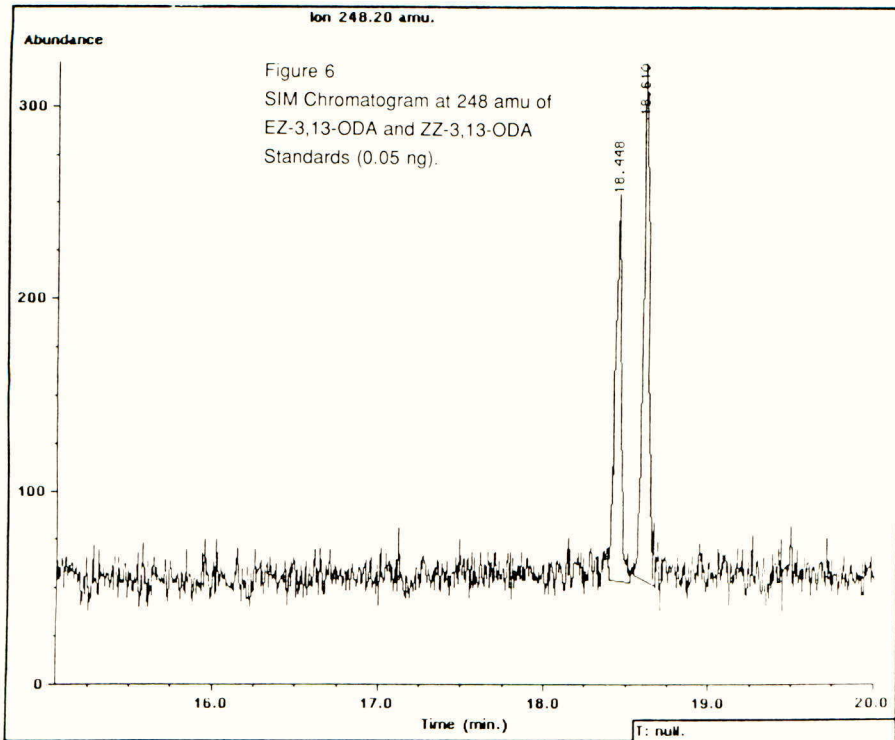
<u>PHEROMONE</u>	<u>COMMODITY-VARIETY</u>	R.T. (min)	SPIKE (ppb)	RECOVERY (%)	MINIMUM (ppb)*
Z-9-DDA	GRAPE-CONCORD	17.7	10,5	98,80	2
Z-9-DDA	GRAPE-SEYVAL	16.3	5	79	2
Z-9-DDA	GRAPE-ELVIRA	16.3	10	88	2
Z-11-TDA	GRAPE-CONCORD	22.7	10,5	90,80	2
Z-11-TDA	GRAPE-SEYVAL	21.2	5	74	5
Z-11-TDA	GRAPE-ELVIRA	21.2	10	89	5
Z-11-TDA	APPLE-MCINTOSH	21.2	10,5	100,89	2
E-11-TDA	APPLE-YORKING	20.9	10,5	106,124	5
Z-11-TDA	APPLE-YORKING	21.1	10,5	92,104	5
Z-11-TDA	APPLE-ROME	---	10,5	---	---
E-11-TDOH	APPLE-YORKING	---	10,5	---	---
E-11-TDOH	APPLE-ROME	---	10,5	---	---
Z-9-DDA	APPLE-YORKING	16.3	10,5	87,116	2
EE-8,10-DDOH	APPLE-GOLDEN DELICIOUS	---	10,5	---	---
DDOH	APPLE-GOLDEN DELICIOUS	---	10,5	---	---
TDOH	APPLE-GOLDEN DELICIOUS	---	10,5	---	---
Z-11-TDOH	APPLE-GOLDEN DELICIOUS	---	10,5	---	---
E-11-TDOH	APPLE-GOLDEN DELICIOUS	---	10,5	---	---
Z-11-TDOH	APPLE-WINE SAP	---	10,5	---	---
E-11-TDOH	APPLE-WINE SAP	---	10,5	---	---
EZ-3,13-ODA	PEACH-ENCORE	18.4	10,5	78	5
ZZ-3,13-ODA	PEACH-ENCORE	18.6	10,5	80	5

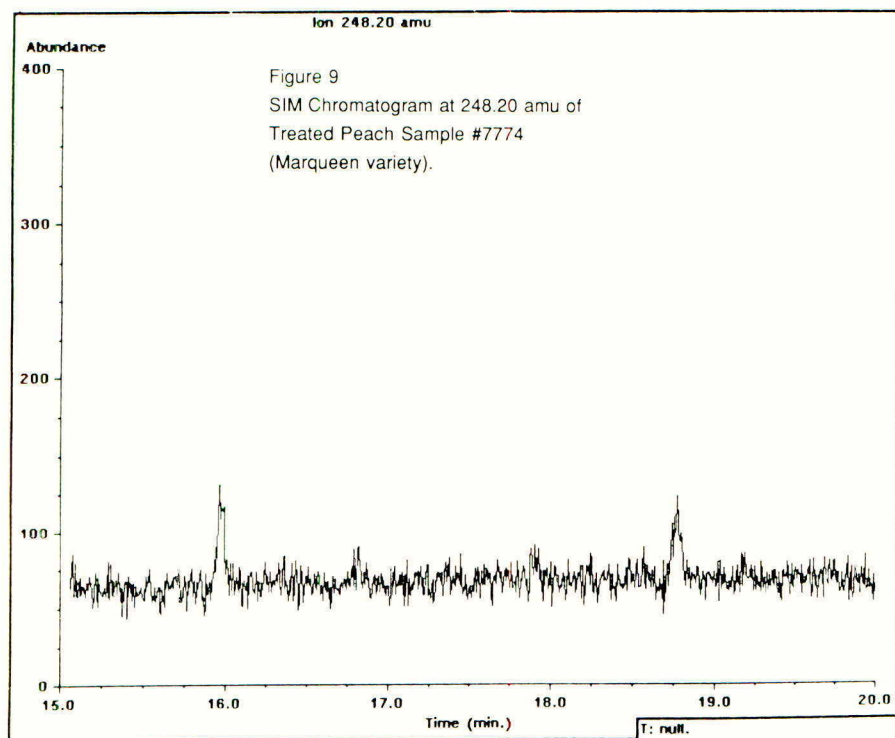
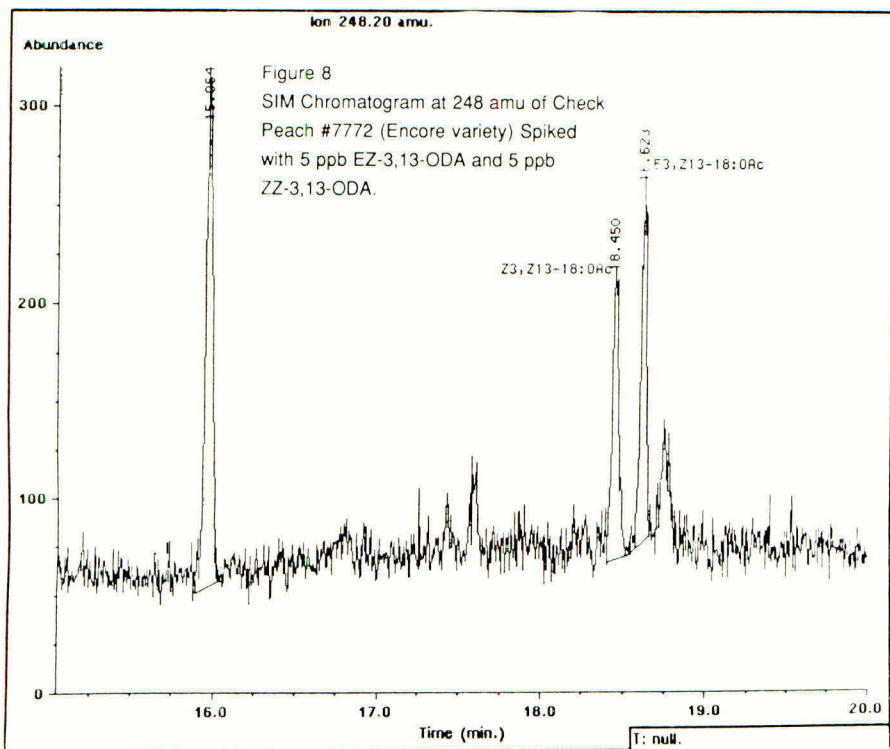
*Minimum measurable concentration

---Data Incomplete









DISCUSSION

Inspection of Tables 1-8 reveals that there have been no detectable residues of any applied pheromone acetate found on any treated sample. This is in agreement with results of our 1988 study. Further, in light of the use pattern of these pheromones (discrete point source dispensers), the results are not at all unexpected considering the highly unlikely probability of any measurable amount of volatilized pheromone selectively condensing on the edible portion of the biomass in an orchard or vineyard. Even a uniform residue distribution of applied pheromones over the entire canopy floor and foliage is a highly improbable event--it would be like attempting to store a few micrograms of a volatile chemical for six months in an open beaker.

The work is incomplete; because the less volatile alcohols are still to be determined, the argument exists that residues may still be found. However, with the exception of EE-8,10-DDOH, all alcohols in this study are 10% or less of the formulation--thus diminishing the likelihood of their being found as residues by a factor of ten.

With increased resources and effort we could confidently extend our sensitivity for these analytes down to the ppt (pg/g) range. This claim could be made for almost any chemical pesticide under scrutiny--frequently it is important that it be done. But, excessive outlays for pheromone residue schemes are probably not the best use for research funds, nor do pheromone residues in this range (if present at all) constitute a significant threat to human or animal health.

A review of lepidopteran pheromone toxicology shows that most pheromone components show no adverse effects up to the NOEL (No Observable Effect Level), a number generally dictated by the maximum amount that the test system (animal) can physically accommodate (Kirsch, 1988). It is less a matter of toxicological response, than an illustration of an exclusion principle. But, they are not inert substances, and they would be expected to undergo common biological oxidations to long chain carboxylic acids (i.e. fatty acids). Plus, there is nothing obvious in the structure of any of the pheromone components in this study that would disqualify them from metabolism by beta oxidation and complete mammalian digestion (Ernster et al., 1965; Griffiths, 1965; Nicholls et al., 1964). In fact, in light of today's health concerns, the worst that might be said against potential (or unmeasurable) lepidopteran pheromone residues is that they might be slightly fattening.

So are we wasting our time and money by conducting these studies? Not really. It is important to document the absence of residues under these conditions to backup anticipated requests for tolerance exemptions or waivers of residue data requirements. Plus, studies demonstrating no residues on food (or foliage, soil and adjacent water, for that matter), would go a long way towards justifying requests for relief from non-target organism studies. Finally, a point of caution, these studies are limited to analysis of fruit crops that have been treated with discrete point source formulations of pheromone. The analyses reported in this study demonstrate no detectable residues under this use pattern, however, these results do not suggest that residues would be absent on crops treated with pheromone formulations that were broadcast or sprayed directly onto the fruit. Research examining the potential of residues from broadcast formulations still needs to be undertaken.

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REFERENCES

Charlton, R. Personal Communications.

Ernster, L.; Lee, C. P. (1964). Biological Oxidations. *Annual Reviews of Biochemistry*, 33:729.

Griffiths, D. E. (1965). Oxidative Phosphorylation. In: *Essays in Biochemistry*, P. N. Campbell and G. D. Greville (Eds), pp. 91. Academic Press.

Kirsch, P. (1988). Pheromones: Their Potential Role in Control of Agricultural Pests, *American Journal of Alternative Agriculture*, 3:84-97.

Nicholls, P.; Schonbaum, G. R. (1964). Catalases. In: *The Enzymes*, P. Boyer, H. Lardy and K. Myrback (Eds) 2d Ed., 8:147. Academic Press.

Spittler, T. D.; Leichtweis, H. C.; Dennehy, T. J. (1988). Biorational Control of Crop Pests by Mating Disruption: Residue Analyses of Z-9-Dodecen-1-yl Acetate and Z-11-Tetradecen-1-yl Acetate in Grapes. In: *Biotechnology for Crop Protection*, P. Hedín, J. J. Menn and R. Hollingworth (Eds), ACS Symposium Series, 379:430-436.